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THE MECHANISM OF ANAPHYLACTIC AND ALLERGIC REACTIONS

An Evaluation of the Role of Histamine in Their Production

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IT is somewhat difficult to ascribe with justice the first description of an anaphylactic reaction to one individual. The exact origin of the subject is obscured in the early development of immunologic studies. The use of repeated injections of solutions containing foreign protein into animals and man to induce or transfer immunity brought to light the hypersensitive state. The subject is new. It belongs to twentieth-century medicine. Typical of this period, expansion has been rapid. Measured by the doubtful criterion of number of papers published, progress has been quickly made. By 1909, with the subject still in its infancy, Anderson and Rosenau[3] found some 200 papers worthy of citation when they reviewed the literature. Since 1935, Rackemann[44-53] has mentioned more than 100 papers each year in his annual surveys of reports on allergy. Despite this enormous annual accumulation of written material, the mechanism of the hypersensitive state with its associated anaphylactic reaction is still not fully understood.

The early studies of hypersensitivity are linked with three great schools: a group in Germany headed by von Pirquet[43] and Schick, a group in France led by Richet[54] and an American school. Von Pirquet and Schick studied the hypersensitive state in human beings and their observations did much to clarify the subject. The continued use of their tests for tuberculosis and diphtheria is testimony of the accuracy of their research. It was von Pirquet who suggested the term "allergy," which is derived from the Greek words *allos* (change) and *ergon* (reaction), "changed reaction," which is still the most direct definition we have for the hypersensitive state. Richet in France, carried out many of his studies on the dog. Nevertheless, it is difficult to find a description of canine anaphylaxis

among his papers. Many of Richet's publications seem diffuse and at times difficult to follow, with a generous proportion devoted to the development of an elaborate terminology which he used to explain allergic phenomena. Richet introduced the term anaphylaxis, which he derived by compounding the two Greek words *ana* (against) and *phylaxis* (protection), "against protection." He thus defined the hypersensitive state as the opposite to prophylaxis. The extent to which this interpretation may be true is still unsettled.

In America, most of the early studies were made on the guinea pig and were concerned with what may be termed the mechanics of anaphylaxis. Such men as Anderson and Rosenau, Gay and Southard[28] established the procedure to be followed in order to induce the hypersensitive state. They showed that the single or repeated administration of foreign protein is followed some days or weeks later by a hypersensitive period during which reinjection of the protein (shock injection) produces a rather stereotyped response typical for each species of animal and independent of the protein used.

By 1909 the basic framework of anaphylaxis was established and a new chapter in immunologic reactions was in the process of being written. At about this time some investigators turned their interest from strictly immunologic studies to the elucidation of the mechanism by means of which the symptoms of anaphylactic shock are produced. To accomplish this they used physiologic methods of investigation. The animals most thoroughly studied from this point of view have been the guinea pig, the dog and the rabbit.

The formation of a toxic chemical substance during anaphylactic shock was one of the first mechanisms suggested for the production of the symptoms of the reaction (Biedl and Kraus, 1909).[9] That this chemical factor might be histamine was first inferred by Dale and Laidlaw[19] in 1910, when they pointed out the similarity between the phenomena of anaphylactic shock and the physiologic action of histamine. Since the publication of their paper considerable evidence has accumulated in favor of the view that many of the symptoms of anaphylactic shock are due to the liberation of histamine.

ANAPHYLAXIS IN THE GUINEA PIG

While Ehrlich, the great German bacteriologist, was visiting America in 1904, Theobald Smith told him that guinea pigs became sick and died when given repeated injections of horse serum. On his return to Germany, Ehrlich gave the problem to Otto (see Anderson and Rosenau. 1909).[3] Otto[42] established the essential features of the reaction and called it the Theobald Smith phenomenon. The term is seldom heard in discussions of anaphylaxis in America today. The predominating symptom of the reaction which Otto described was progressive respiratory difficulty identical with that observed in histamine poisoning in the guinea pig. Gay

and Southard in 1908, pointed out that in fatal reactions respiration ceased in the inspiratory phase and that the lungs were emphysematous. Auer and Lewis[5] in 1910, demonstrated that the respiratory difficulty was caused by contraction of the smooth muscle of the bronchioles. The condition might be termed asthma in the guinea pig. It is exactly duplicated by the injection of histamine into this animal.

Schultz[63] in 1910, in America, and Dale[18] in 1913, in England, demonstrated that isolated perfused uteri from sensitized guinea pigs contracted forcefully upon addition of the sensitizing antigen. The response of the smooth muscle was identical with that given by histamine.

Utilization of the isolated perfused organ technique has recently given more direct evidence of the liberation of histamine during anaphylactic reactions in the tissues of the guinea pig. Bartosch, Feldberg and Nagel[7] in 1932, showed that a histamine-like substance was liberated into the perfusate when the antigen was administered to the isolated perfused lungs of sensitized guinea pigs. Schild, in 1937[61] and 1939[62], confirmed this observation and extended the study to other organs of the guinea pig. He found that histamine was liberated in readily estimable quantities from the isolated aorta, uterus, liver and lungs of sensitized guinea pigs when these tissues are exposed to the antigen. Schild's experiments also suggest that the contraction of the uterus of sensitized guinea pigs observed by Schultz[63] and Dale[18], is due to the liberation of histamine following a reaction between the tissue of the uterus and the antigen. He found, rather oddly, that smooth muscle taken from the gastro-intestinal tract of sensitized guinea pigs did not contract on administration of antigen nor did it show liberation of histamine. A further study of this difference between the smooth muscle of the gastro-intestinal tract and the smooth muscle of the aorta and uterus might yield interesting information.

In 1935 Barsoum and Gaddum[6] introduced a quantitative procedure for the estimation of histamine in the blood. Experience with this method led to its modification and the evolution of a somewhat simplified procedure (Code, 1937).[12] The method has given an accurate and highly sensitive tool for the further study of anaphylactic reactions. The advances accomplished by its use have been sufficient to justify their somewhat detailed examination.

The amount of histamine in the blood of animals varies widely in different species. Of the blood of normal animals so far tested, rabbit blood has consistently shown the highest concentration, with values usually ranging from 1 to 2.5 micrograms of histamine base per cubic centimeter of blood. The blood of the guinea pig comes next in line with a histamine content of approximately 0.05 to 0.15 microgram per cubic centimeter. Dogs' blood consistently contains little or no histamine. The blood of man falls between that of the guinea pig and that of the dog with amounts usually ranging from quantities which can just be detected up to about 0.06 microgram per cubic centimeter of blood.

When dealing with a substance possessing such potent physiologic properties as histamine, an important question to answer is: Is the histamine free in the blood to produce its physiologic effects or is it held safely within the cells of the blood? Because of the relatively high con-

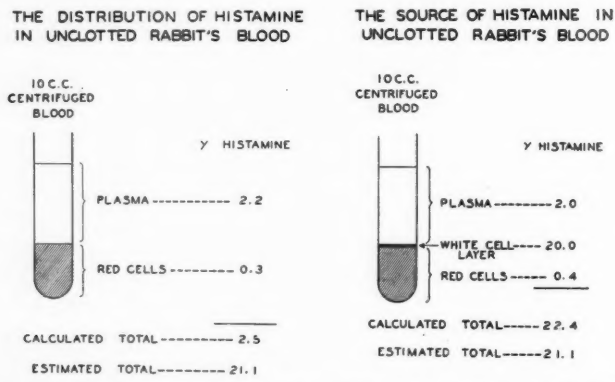

Fig. 1. *(left)* The distribution of histamine between red cells and plasma in normal rabbits' blood. The amounts of histamine in the plasma and red cells together accounted for only 12 per cent of the total found to be present in 10 c.c. of the whole blood not centrifuged.
Fig. 2 *(right)* The source of histamine in normal rabbit's blood. When the white cell layer which separates out between plasma and red cells was included in the histamine estimations, it was found to be the major source of the histamine in the blood.

centration of histamine in rabbit's blood, the distribution of the histamine between cells and plasma was first studied in detail in this animal (Code).[13,14] An example of experiments carried out on blood from rabbits will serve to illustrate the consistent findings.

Ten cubic centimeters of heparinized unclotted rabbits' blood was centrifuged. The plasma was found to contain 2.2 micrograms of histamine and the red cells contained even less. Ten cubic centimeters of the same blood not centrifuged contained 21.1 micrograms of histamine. The plasma and red cells together accounted for only 12 per cent of the total amount of histamine present in this sample of blood (Fig. 1). The only portion of the centrifuged blood not studied in this sample was the layer which separates out between the plasma and red cells and contains the leukocytes and platelets—"the white cell layer" or buffy coat. When this layer was included, the missing histamine was recovered. The white cell layer was found to be the major source of the histamine contained in blood. In this example the white cell layer contained 89 per cent of the whole blood histamine (Fig. 2). Using this information, Ing and I in 1937[16], isolated histamine in pure crystalline form from the white cells of rabbit blood.

The results have been extended to include man, the horse, the bullock, the goat, the calf and the dog. In all these species, 70 to 100 per cent of the blood histamine was found in the white cell layer of centrifuged blood

TABLE I. HISTAMINE CONTENT OF THE BLOOD OF GUINEA PIGS BEFORE AND DURING ANAPHYLACTIC SHOCK AND NITROGEN ANOXEMIA

Guinea pig	Conditions	Histamine per c.c. blood, micrograms	
		Before shock	During shock
1	Anaphylactic shock	0.150	0.600
2	Anaphylactic shock	0.127	0.959
3	Anaphylactic shock	0.166	1.250
4	Anaphylactic shock	0.080	0.779
5	Anaphylactic shock	0.184	0.685
6	N_2 anoxemia	0.143	0.118
7	N_2 anoxemia	0.149	0.141
8	N_2 anoxemia	0.118	0.066

(Code, 1937).[13] In general these results have been confirmed by Minard[40] and by Rose[56], and Rose and Weil.[60] Zon, Ceder and Crigler[69], and Minard[40], have found that the platelets of rabbits' blood may contain histamine. It may be concluded that in most species under normal conditions, the white cell elements are the source of the histamine in normal blood.

To return to anaphylactic reactions, the method has been used to determine the changes in the histamine content of the blood of guinea pigs and during anaphylactic shock (Code, 1939).[15] The animals were sensitized to egg white and horse serum. Control samples of blood were withdrawn from the sensitized guinea pigs by cardiac puncture and through the same needle the antigen was immediately injected. When respiratory difficulty was maximal, anaphylactic blood was taken by transection of the vessels of the neck. The results are illustrated in Table I. The first five animals of this series were in a state of anaphylactic shock from which recovery seemed most improbable when the final blood sample was taken. The histamine content of the blood had risen from three to nine times its control value.

Since the guinea pig in severe anaphylactic shock suffers from an extreme degree of oxygen lack, it was possible that the anoxemia alone might account for the increased amounts of histamine in the blood. To check this possibility, a number of animals, of which the last three in Table I are representative examples, were placed in an atmosphere of pure nitrogen. Blood samples were taken before exposure to nitrogen and when respiratory movements had ceased as a consequence of exposure to the nitrogen. The blood histamine was, if anything, reduced.

It may be concluded that in severe anaphylactic shock in the guinea pig the histamine content of the blood is increased and that this increase is not due to the coincident anoxemia.

The results are in accord with the earlier studies on isolated perfused

organs. On the basis of Schild's[61] experiments, it seems likely that the increased quantity of histamine found in the blood of the intact guinea pig did not originate from a single organ but arose from tissues scattered throughout the animal. It may be concluded that in the guinea pig histamine is liberated during anaphylactic shock and that it plays a definite role in the symptomatology of the reaction in this species.

ANAPHYLAXIS IN THE DOG

Biedl and Kraus[9] in 1909, first described the two most outstanding features of anaphylactic shock in the dog, the fall of blood pressure and the reduced coagulability of the blood. The fall of blood pressure they thought was due to vasomotor paralysis caused by the formation of a toxic peptonelike substance. Good evidence of liberation of a circulating toxic substance appeared the following year when Manwaring[38] published the results of experiments carried out in the laboratory of the late Professor E. H. Starling at University College, London. Manwaring found by cross-circulation methods, that when a normal dog receives blood from a dog in anaphylactic shock, signs of anaphylaxis develop. In his experiments exclusion of the liver from the circulation of the sensitized dog prevented the occurrence of shock. His data indicated that the acute fall of blood pressure which occurs during anaphylaxis in the dog is due to the explosive liberation of depressor substances from the liver.

The importance of the presence of the liver in anaphylactic shock in the dog was corroborated and emphasized by the studies of Voegtlin and Bernheim[64] in 1911, and of Denecke[20] in 1914, and by a series of investigations by Weil[66,67], and Weil and Eggleston[68] in 1917. The interpretation placed on the hepatic changes by Weil was not that originally stated by Manwaring. Because his experiments did not indicate the presence of toxic factors in the blood, Weil concluded that the fall of blood pressure in canine anaphylaxis was a secondary result of hepatic congestion. In the light of more recent findings, Weil's failure to demonstrate toxic substances in the blood seems most certainly to have been due to the methods used for detection. For similar reasons Manwaring and his collaborators[39] in 1925, were unable to detect depressor substances in blood from the carotid artery of dogs in anaphylactic shock. Nevertheless, in their experiments the blood from the liver did possess definite blood pressure lowering properties.

The problem rested in this somewhat confused state until the outstanding studies of Dragstedt and his associates[21,22,23,24,29] were reported a few years ago. They demonstrated the presence of a vasomotor substance in blood taken from the inferior vena cava just above the diaphragm. The site is important because the vein in this locality carries a good deal of blood which has come directly from the liver. Extensive investigation of the active substance by Gebauer-Fuelnegg and Dragstedt[29] in 1932, and by Dragstedt and Mead[22] in 1936, allowed them to

conclude that it was histamine. Like Weil[67], and Manwaring and his associates[39], Dragstedt and Gebauer-Fuelnegg[21] did not consistently find a vasomotor substance in blood from the femoral or carotid vessels.

The development of the Barsoum-Gaddum method for the quantita-

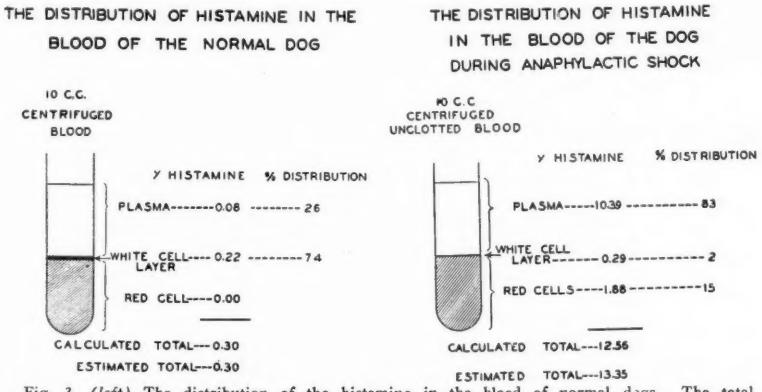

Fig. 3. *(left)* The distribution of the histamine in the blood of normal dogs. The total amount of histamine present is small, being only 0.3 microgram per 10 c.c. There is little histamine—indeed, often none—in the plasma. The white cell layer which separates out between the plasma and the red cells contains 74 per cent of the total present and is consistently the major source of the histamine in the blood of normal dogs.

Fig. 4. *(right)* The histamine in the blood of dogs taken during the early stages of a severe anaphylactic reaction. The total amount of histamine present is increased, being 13.4 micrograms per 10 c.c. The white cell layer has practically disappeared and the remnant accounts for only 2 per cent of the total present. The plasma contains 83 per cent of the histamine present and is thus the major source of the histamine in blood drawn under these conditions.

tive estimation of histamine in the blood has facilitated the investigation of this problem. The refinements accomplished by the method have allowed the estimation of quantities of histamine which would escape detection by earlier procedures. Using this technique the histamine concentration of the peripheral blood has been followed during anaphylactic shock of varying severity in more than twenty dogs (Code, 1939).[15]

Whenever the typical signs of anaphylactic shock developed, the concentration of histamine in the blood was increased from two to more than eighty times the control value. The rise in the blood histamine roughly paralleled the degree of shock displayed by the animal—being greatest when the reaction was severe and least during mild reactions.

In blood from normal animals most of the histamine is held within the white cells (Fig. 3). Before ascribing any of the symptoms of anaphylaxis to the increased quantities of histamine present in the blood, it was important to determine whether the additional histamine was in the plasma, free to produce its physiologic effects, or was held safely within the cells of the blood. The distribution of the histamine in centrifuged blood taken during the early stages of severe anaphylactic reactions was studied. The results were in striking contrast to those obtained with normal blood. The white cell layer had practically disappeared from the blood.

Some of the histamine had diffused into the red cells but its major source was the plasma, where it was free to produce its physiologic effects (Fig. 4).

In most experiments the histamine content of the blood was determined

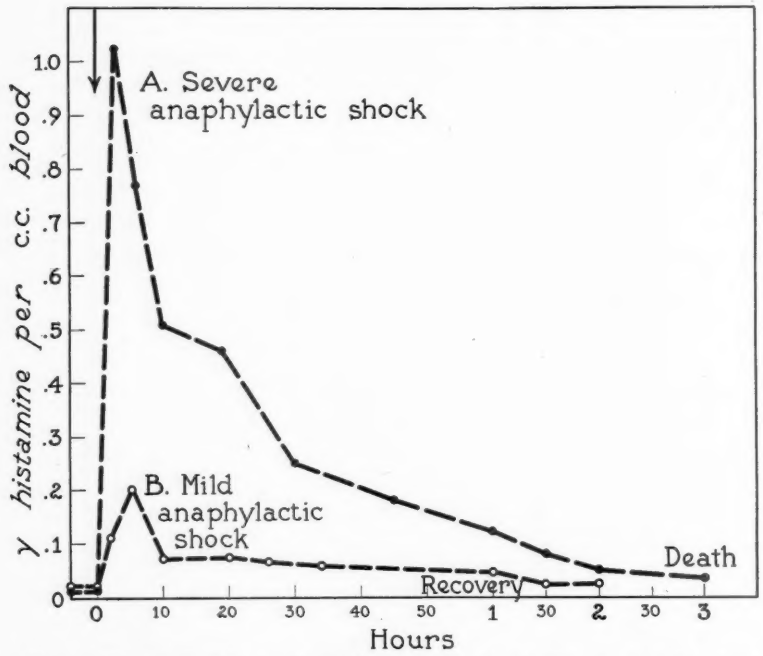

Fig. 5. (From C. F. Code, Am. J. Physiol., 127:78-93, [Aug.] 1939. See page 83.) Blood histamine changes during a mild and a severe anaphylactic reaction in anesthetized dogs. At the arrow the antigen was given intravenously.

at frequent intervals for periods of one, two or three hours or more. The maximal concentration of histamine was generally reached within ten minutes after injection of the shock dose of antigen. After this initial rise the concentration fell rapidly, often returning to normal values in two to three hours (Fig. 5).

It was noted that this explosive liberation of histamine coincided with the dramatic fall of blood pressure which is such a prominent feature of anaphylaxis in the dog (Fig. 6). The amounts of histamine present in the blood were sufficient to account for the fall of blood pressure. The correlation between the fall of blood pressure and the rise of blood histamine was striking but it should not completely overshadow the observation that if the animal survived this initial period, the blood histamine quite rapidly returned to normal levels.

In my experience dogs die of anaphylaxis in either of two stages of the

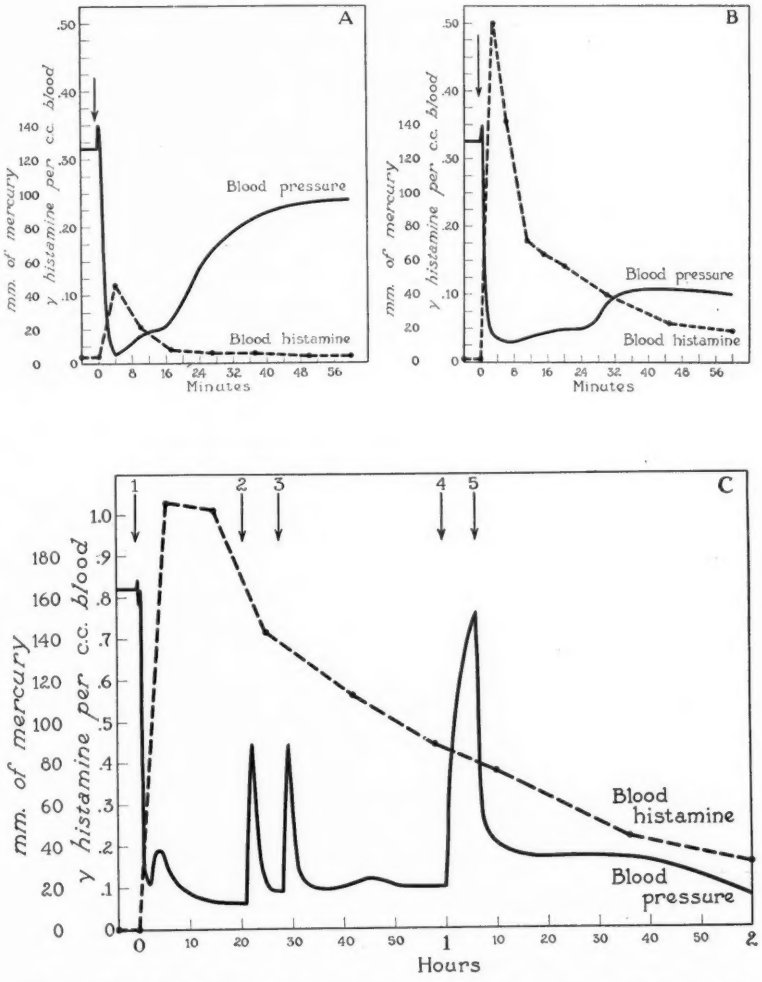

Fig. 6. (From C. F. Code, Am. J. Physiol., 127:78-93 [Aug.] 1939. See page 87.) The blood pressure and blood histamine changes during anaphylactic shock in anesthetized dogs. At the first arrow in each experiment the antigen was injected intravenously. *A*, mild, anaphylactic shock; *B*, a more severe reaction; *C*, fatal reaction—seventeen minutes after injection of antigen the animal ceased breathing and blood pressure was less than 20 mm. of mercury; artificial respiration was commenced. At arrows 2 and 3 and between arrows 4 and 5, epinephrine and 10 per cent solution of glucose were given intravenously. With the continued high blood histamine these supporting measures were ineffective, the blood pressure did not increase and the animal succumbed a few minutes after the two-hour mark.

reaction. When the amounts of histamine liberated are sufficient, the circulation is flooded with this highly active capillary dilatant, in the presence of which the blood pressure cannot recover and death occurs early in the chain of events (Fig. 6*C*). The animal may, however, recover from this early phase of the reaction, the blood histamine returning to

normal values and the blood pressure recovering somewhat. Later some animals even with a normal blood histamine may sink into profound shock and coma and die with a normal blood histamine. While histamine seems quite clearly to be the cause of death in the early stages of the reaction, there is some difficulty in incriminating it as the lethal factor when its presence can barely be detected in the blood.

Also, while histamine can account for the sudden fall of the blood pressure during acute anaphylaxis in the dog, it does not account for the development of incoagulability of the blood, which is such a striking feature of the reaction in this species. Jaques and Waters[32] in 1941 showed that the blood fails to clot because heparin is liberated from the liver during the reaction. The preponderance of evidence definitely indicates that most of the histamine released during the reaction likewise comes from the liver (Watanabe[65], 1931; Ojers, Holmes and Dragstedt[41], 1941). It seems probable that histamine and heparin are liberated simultaneously as a result of a reaction or disruption within the hepatic cells.

The data suggest the following interpretation: first, that histamine is not the fundamental factor in anaphylaxis or in allergic reactions and that a deeper search into the etiology of this baffling condition must be made; second, that histamine is liberated as a consequence of damage done within the sensitized cells, and it is this damage to the cell that is the fundamental etiologic factor in allergic or anaphylactic reactions. Liberation of histamine may be purely incidental. If the damaged cell happens to contain histamine, it will be liberated during the reaction. It is unlikely that histamine produces the damage. Liberation of histamine may be a dramatic and indeed lethal factor if the quantities liberated are sufficient and if, as in the guinea pig, the animal is sufficiently sensitive to its effects. But, as has been pointed out, dogs may die hours after the increase of histamine in the blood has disappeared. Such animals, it seems likely, die as a consequence of the damage which, incidentally, liberated the histamine. Histamine is not the only substance liberated. In allergic reactions in the dog there are symptoms not only of histamine poisoning but also of heparin action. It seems probable that the fundamental mechanism of anaphylactic reaction lies in the process producing the damage within the cell, as a consequence of which these substances are liberated.

ANAPHYLAXIS IN THE RABBIT

Severe anaphylactic shock in the rabbit is accompanied by a fall of carotid blood pressure. Auer[4] in 1911 emphasized the role of the heart in the fall of the blood pressure. He noticed the pronounced dilatation of the right side of the heart during fatal reactions and he concluded that failure of the heart was the cause of acute lethal anaphylaxis in this animal. The work of Airila[2] in 1914, Coca[11] in 1919 and Drinker and Bronfenbrenner[27] in 1924 has clearly shown that during acute anaphylaxis

in the rabbit there is a pronounced increase of peripheral resistance in the pulmonary vascular bed due to contraction of the muscular coat of the pulmonary arterial tree and that this is the cause of the right-sided heart failure. Histamine injected into rabbits also causes pulmonary vascular constriction (Dale and Laidlaw[19], 1910; Cloetta and Anderes[10]; Rocha e Silva[55], 1940). Is the pulmonary vascular constriction of acute anaphylactic shock in the rabbit produced by histamine?

Rose and Weil[60] in 1939 and Rose[56] in 1940 showed that the blood histamine falls in anaphylactic shock in the rabbit. Can a reduction in the blood histamine possibly contribute to the symptoms of the reaction? The important question is: Where is the histamine in the blood—is it in the cells or in the plasma? In normal rabbit blood the major amount is present in the white cell elements. In severe anaphylaxis leukopenia uniformly occurs. In perfusion experiments using lungs and blood from sensitized rabbits, Dragstedt and associates[25,26] have demonstrated that on addition of the sensitizing protein to the blood, 50 per cent of the white cells drop out of the circulation during their first passage through the lungs, and at the same time the histamine content of the blood is reduced (Dragstedt, Ramirez de Arellano and Lawton[25], 1940; Dragstedt, Ramirez de Arellano, Lawton and Youmans[26], 1940).

What happens to the histamine in these white blood cells during the reaction? The white blood cells of the rabbit are rich in histamine (Code and Ing[16], 1937). Can they, like other cells, participate in the reaction? Katz[33] in 1940 showed that the cells of sensitized rabbits' blood do react. He withdrew blood from sensitized rabbits, added the antigen and incubated the mixture for twenty minutes. The plasma from this "shock" blood contained two to six times as much histamine as plasma from the same blood tested in a similar fashion but to which no antigen had been added. Dragstedt and co-workers in 1940[25] and 1941[30] and Rose [57] in 1940 confirmed this observation. Rose has given a good example of the experiment.[57] In a control sample of blood from a sensitized rabbit, 85 per cent of the histamine was in the white cell layer. In the "shock" blood from the same animal incubated with antigen 80 per cent was found in the plasma. As Abell and Schenck[1] showed in 1938, the physical properties of white blood cells are altered in rabbits during anaphylactic reactions. In the test tube, during the shock reaction the white cell elements of the blood are altered or damaged and their histamine is released into the plasma.

Although the whole blood histamine may fall during anaphylaxis in the rabbit, the amount liberated from the white cells would be sufficient to produce a marked constriction of the pulmonary vessels. Again in this species it seems that histamine produces the pronounced and often lethal symptom of the reaction, but again cells have been damaged and it is suggested that search into this cellular disruption must be made if the fundamental factors in anaphylaxis are to be unearthed.

COMMENT

Some profit may be derived from a discussion of certain features of allergic reactions of human beings in the light of facts and conclusions derived from animal studies. Here I must tread lightly because my experience is limited and the clinically trained reader is much better fitted to lead one into this maze than I.

First, what are the routes through which a sensitizing agent may normally enter the body? They are (1) the skin and conjunctiva; (2) the upper portion or the pulmonary portion of the respiratory tract and (3) the gastro-intestinal tract. Once the agent has passed these surface structures, it will be in the blood stream and thence may reach any of the body tissues.

What tissues of the body commonly display allergic reactions? There seems little doubt that allergic reactions are most commonly observed in the tissues listed at the external surfaces of the body, the tissues which since man's beginnings have been brought in contact with sensitizing agents. They might be termed the barrier tissues—barring the way of sensitizing agents to deeper, more vital structures. All of the surface tissues display allergic reactions. All so far tested contain histamine (Best and McHenry[8], 1931). Does histamine contribute to the symptoms produced in an allergic reaction in these tissues?

Histamine has three major physiologic actions. It acts (1) on smooth muscle to produce contractions; (2) on capillaries to produce dilatation and increased permeability, which may lead to formation of edema and (3) on secretory glands as a secretagogue. The acute phases of allergic reactions in many tissues seem adequately explained on the basis of liberation of histamine. In the lungs, for example, histamine acting on smooth muscle to produce bronchiolar constriction, on capillaries to produce edema and on the mucus glands to produce mucus, could quite accurately reproduce the findings of asthma. But the evidence is purely indirect.

In the skin, the urticaria and wheals of an acute reaction may also be duplicated by injection of histamine (Lewis and Grant[37], 1924). More direct evidence, however, is available. Katz[34] in 1942 devised a method whereby he can test for the liberation of histamine during local allergic reactions in the skin. With patients showing a skin reaction to the intradermal injection of ragweed, he observed a sharp increase of output of histamine from the skin into which the antigen had been injected. The studies of Rose[58] in 1941 on the blood of patients suffering from dermographia and cold allergy have shown that during formation of extensive wheals histamine may be liberated from the skin and appear for brief periods in increased quantities in the blood. Once again histamine is associated with the acute phases of the allergic reaction. Its role at the surface of the body may be the production of a wheal in an attempt to limit the further entrance of the sensitizing agent. But what of the fate

of the damaged surface cell from which the histamine was liberated? Is it not possible that in certain types of chronic dermatitis we observe this battered cell—so often damaged by repeated allergic onslaughts that it bears little resemblance to its former self, so often damaged that it can no longer give standard cutaneous reactions?

Of the tissues within the body, the blood is by far the most accessible and as a consequence has been the most carefully studied. Haworth and MacDonald[31] in 1937 compared the concentration of histamine in the blood of seventy-five card-room workers suffering from cotton-dust asthma with that in the blood of 103 university students and eighteen persons suffering from chronic bronchitis. The concentration of histamine in the blood of the asthmatic group was normal or slightly greater than normal, with an average value for the group a little greater than that of the university students and the chronic bronchitic patients. Repeated observations were made in a number of instances on the students and on the asthmatic patients. The histamine content of the blood of the normal students remained remarkably constant while that of the asthmatic patients showed marked fluctuations.

Rose[59] in 1941 confirmed these findings and extended them to other allergic conditions. His series included patients suffering from asthma, urticaria, angioneurotic edema, eczema and vasomotor rhinitis. The concentration of histamine in the blood of these patients was usually normal or slightly elevated. In some cases, during acute attacks there was a definite reduction of the blood histamine level, particularly in cases of angioneurotic edema. With the exception of patients having urticaria, all tended to have fluctuating blood histamine values compared with the constant levels noted among normal subjects. It seems likely that the histamine content of the blood of patients suffering from these allergic conditions is usually within the normal range but it tends to fluctuate widely in contrast to the constant values usually found among normal persons. The most that can be said from these data is, it seems, that in certain allergic conditions histamine metabolism is disturbed.

But the whole blood histamine is simply the reflection or the mean of a variety of possible changes in cells containing histamine scattered throughout the body. The blood itself contains such cells. Katz and Cohen[35] in 1941 showed that the cells of the blood of patients sensitive to ragweed, timothy or horse dust liberate histamine when exposed *in vitro* to the specific allergen. The blood cells of the allergic human being are similar in this respect to those of the sensitized rabbit, guinea pig and dog (Katz, 1940).[33] Apart from blood and skin cells, do cells elsewhere in the body liberate histamine during allergic reactions? There is no direct evidence that this occurs in man.

The red bone marrow contains histamine (Code and Jensen, 1941)[17] and it may be involved in the reaction of serum sickness (leukopenia, agranulocytosis). But tissue need not contain histamine to react. The

central nervous system, particularly the brain, contains little or no histamine (Kwiatkowski, 1943)[36]; yet it most certainly may be involved in severe reactions.

CONCLUSIONS

Thus, for the present the following conclusions may be tentatively drawn:

1. Histamine is released during acute allergic reactions as a consequence of damage to cells already containing histamine.

2. Its release from such cells is rapid and explosive and the histamine may be in sufficient quantity to kill the animal. However, if the animal survives, the histamine soon disappears. When the histamine has disappeared, there may still remain the damaged cell.

3. Cells which do not contain histamine may suffer damage as a consequence of an allergic reaction and the damage may produce symptoms—even death.

4. Thus, in allergic reactions histamine may produce a dramatic and often distracting veil of symptoms behind which lies the damaged cell. The mechanism of that damage to the cell is the challenge to allergists today.

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THE MECHANISM OF DESENSITIZATION

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TYPICAL anaphylaxis occurs only under artificial circumstances, when relatively large doses of antigen are willfully introduced at a relatively rapid rate, more or less directly into the circulation of animals sensitized by the parenteral exposure to the same antigen, many (not less than seven or ten) days previously.

If, instead, these animals receive a relatively small test dose of antigen, or even if a larger dose is given in high dilution or injected slowly,† or if the injection is made only a short time (not more than three to four days) following initial (or the last serial) exposure, the acute anaphylaxis does not occur, although (depending on the size of the test dose) the animals may show characteristic symptoms of subacute anaphylaxis of varying degrees of severity. Moreover, such animals, after a prompt recovery, may now withstand the rapid injection of several times the test dose of antigen which would have certainly killed them if it were so given in the first place.[2,3]

This refractory state, called a "state of antianaphylaxis" by Besredka, is only relatively effective (it may be overcome at any stage by increasing the test dose of antigen). This refractoriness lasts for a relatively short time (hours or days), depending on the amount of antigen injected, after which the reactivity returns; that is, the animal is again capable of responding to small doses of specific antigen with the original intensity.

At first glance this temporary refractoriness might suggest that "sensitivity" of the animal has decreased or has been abolished and, because this refractory state was produced by injection of specific antigen, it looks as though the antigen has combined with or exhausted the available antibody. It is for this reason the phenomenon is currently referred to as "desensitization."

This term is highly misleading and has been instrumental in obscuring the true facts by suggesting (as many investigators hold even today) that the level of antibody determines the type of the reaction of the animal to the antigen. In fact, Topley and Wilson[49] summarize this view as follows:

"The balance of experimental evidence is strongly in favor of the view that the anaphylactic response is the result of an antigen-antibody reaction occurring on or in the cells that have removed the antibody from the blood and in some way fixed it themselves. The anaphylactic state is associated with the presence of

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†Lewis, J.A.M.A., 76:1342, 1921.

fixed antibody and the absence of circulating antibody. The immune state is associated wtih the presence of circulating antibody in a concentration sufficient to protect the fixed antibody that is also present."

Similarly, Wells[52], in discussing the nature of the antianaphylactic state, says: "This term should logically be applied only to a resistance due to antibodies." In short, according to this concept, when test dose of antigen is injected into the animal possessing an excess of circulating antibody, the latter combines with the antigen and prevents it from reacting with cellular antibodies, and thus the tissue injury and resulting symptoms are prevented.[22]

While it is true that guinea pigs may be rendered hypersensitive (to some antigens at least) by a single, relatively small preliminary injection, presumably resulting in the production of limited amount of antibody, this is not true generally. For instance, in order to elicit any signs of hypersensitiveness in rabbits, dogs, and particularly in monkeys, these animals must receive many injections of antigen over a period of many days "until considerable amount of antibody has appeared in the blood[55]," and they do not exhibit symptoms of hypersensitiveness while antibody content is low. Similarly, man is relatively infrequently sensitized by a single exposure to antigen; as a rule hypersensitiveness develops only as a result of repeated exposures continued over a relatively long period of time.

Furthermore, if it were true that excess of circulating antibody protects the sensitized tissue, it should follow that through repeated injection of antigen over a period of hours (as it is done to prevent shock in connection with serum therapy in hypersensitive individuals), more and more of the circulating antibody should be bound and thus, at the later stages of this process, the tissues should be more and more likely to react with the antigen. Actually, the reverse is true; the more antigen is injected (in gradually increasing amount), the more solid is the resistance to shock from the subsequent injections of antigen.

It is true that under different circumstances (as in the attempts to free the patient suffering from hay fever of their symptoms) where patients receive a number of injections of antigen continued over a long period of time, they actually *do* develop additional amounts of circulating antibodies by the time they become refractory ("desensitized"). It is commonly stated that these previously "hypersensitive" individuals are made "immune" (do not suffer from exposure to the allergen), but the evidence that this freedom from symptoms is directly referable to the acquisition of additional antibody is, to say the least, not convincing. Indeed, if production of an excess of antibody were the essential (necessary and sufficient) means of producing refractoriness (or antianaphylactic state), how could one account for the fact that previously hypersensitive animals become antianaphylactic immediately following injection of a single large (but sublethal) dose of antigen (as in guinea pigs), or immediately following the completion of a series of injections of small

(increasing) amounts of antigen given within a few hours (as in the case of human beings hypersensitive to therapeutic serum). These procedures certainly do not involve the immediate production of additional antibody. In fact, the production of antibody following these injections becomes detectable only several days later; but by the time additional antibody actually appears, the state of refractoriness has disappeared and the original hypersensitiveness has usually returned.

That high concentration of circulating antibody does not protect the animal against anaphylactic shock is best illustrated by the well-known observation that immune animals with high antipolysaccharide antibody content in their blood (so high that a fraction of a cubic milliliter of their serum will passively protect mice against many thousand lethal doses of pneumococcus), are killed by anaphylactic shock if a mere trace of specific polysaccharide in solution is injected intravenously.

The erroneous notion that excess of circulating antibody protects the animal against anaphylaxis by preventing the antigen from reaching the sensitized tissues has its origin in the experiments of Friedberger[22], further strengthened by Weil.[51] This author found that injection of additional antibody into the circulation of previously sensitized guinea pigs rendered them refractory to antigen.[51] However, his conclusions as to the mechanism of this refractoriness have since been found to be wrong. As found in my laboratory by Dr. Morris[37,38], the refractoriness of animals in Weil's experiments was due, not to excess of antibody as such, but to the fact that he introduced the antibody in the form of foreign (rabbit) serum.* The effect would have been exactly the same if, instead of immune rabbit serum, he would have injected normal rabbit serum (or even more effectively, normal fowl or horse serum) immediately preceding the test injection of antigen.[20,27,31] Our own unpublished experiments (1915), later confirmed by Morris[38], have shown that if the antibody is derived from a homologous animal, if the antibody is collected late in the process of immunization to avoid the presence in it of traces of antigen, and if from twenty minutes to one hour is allowed to elapse between the injection of additional antibody and the injection of antigen (so as to allow time for a nonspecific "anaphylactoid" effect of injection to be dissipated), the animals receiving additional antibody actually are more hypersensitive than the controls. That is, the anaphylactic shock can be elicited in the former animals with a considerably smaller test dose of antigen than in the latter (controls).

From the preceding discussion, it seems clear that excess of circulating antibody does not produce the state of refractoriness or antianaphylaxis. It is evident, therefore, that the effectiveness of "desensitization" through a properly conducted (single or repeated) exposure to antigenic stimulation must involve some other mechanism.

*Friedberger[23] pointed out still another source of error to be guarded against in such experiments—the additional serum injected into sensitized animals may contain traces of antigen and thus may render recipient antianaphylactic as a result of the reaction between this antigen and the recipient's antibody.

The definition of the basic difference between the state of immunity and that of hypersensitiveness, as quoted from Topley and Wilson[49], suggests at least one other possible mechanism. In fact, in an earlier edition of the book,[48] these authors have specifically stated that, as a corollary to the view that fixed antibody is present in both the hypersensitive and immune animals, and that the latter (immune) owe their lack of hypersensitiveness to the presence of an excess of antibody in the circulating blood, one is justified in inferring that[48],

> ". . . a 'hypersensitive' animal may be desensitized in two ways: by receiving a small dose of antigen, so administered to neutralize the fixed antibody without precipitating acute shock, or by receiving repeated doses of antigen so spaced and graduated as to give rise to a high concentration of antibody in the circulating blood."

There is no doubt that hypersensitive animals can be made refractory to antigen either by a single or by multiple exposure to sublethal (or gradually increasing) doses of antigen. However, we have seen that the production of additional antibody is not the mechanism responsible for this effect.

Let us now consider the second mechanism that has been suggested[48], namely, that hypersensitive animals may be rendered refractory by saturation of the sessile antibodies.

In discussing the "desensitization" as being the result of saturation of cellular antibody, Zinsser[53] suggested that, "the animal so treated (properly desensitized) for a time, loses its capacity to react with antigen in a manner analogous to chemical saturation." This hypothesis is not open to direct verification since we know of no methods for quantitative evaluation of the cellular antibody.

It is quite conceivable that in such animals in whom hypersensitiveness exists without demonstrable circulating antibody, the antigen, which is injected for the purpose of "desensitization," would combine directly with the sessile antibodies and might conceivably, under special conditions, saturate them. However, in such animals in whom excess of circulating antibody is present, no such direct union with cellular antibody can be expected. (In fact, the other half of the hypothesis actually postulated the preferential anchoring of the antigen by the circulating antibody and resulting prevention of its union with cellular antibody.)[48] Thus, if saturation of cellular antibody were essential for establishment of the state of refractoriness, the "desensitization" of animals possessing excess of circulating antibody would be expected to be impossible, or at least more difficult to attain.

Since in actual experience desensitization *does* occur in such animals (and with usual doses of antigen), in spite of the presence of circulating antibody, and since such "desensitized" animals still possess enough free antibody left over (so that a small amount of their serum may sen-

sitize normal homologous animals), it would seem necessary to postulate that, if in "desensitized" animals antigen has united with sessile antibody, the antigen must possess a greater affinity for sessile antibody. Realizing this and other difficulties in trying to correlate respective significance of the cellular and humoral antibodies, Weil[51] actually stated that "the only solution appears to lie in the assumption of an increased 'avidity' of the anchored antibodies, as compared with the free antibodies, for the antigen."

But if this auxiliary hypothesis is accepted, then it is clear that the definition of an immune state[49] as depending on preferential union of antigen with the circulating antibody, thus preventing it from reaching the sensitized tissues, must be abandoned.

In short, the suggestion of a dual mechanism as given by Topley and Wilson[48] for accomplishing "desensitization" is untenable because it contains an internal contradiction—the two mechanisms are mutually exclusive and cannot be offered as component part of one hypothesis.

In considering the second part of the hypothesis by itself—namely, that "desensitization" is due to exhaustion or saturation of the sessile antibody[53], one is confronted immediately with several facts which contradict it. In the first place, no matter how effectively an animal is "desensitized" (presumably because its sessile antibodies have been saturated), such animal can be thrown into anaphylactic shock by an injection of a large dose of antigen. The "desensitization" is never complete, but only relative. If the cells have lost their power to unite with moderate amount of antigen, how can they react with a larger one?

Moreover, when animals are simultaneously sensitized to two or more antigens and receive a "desensitizing" injection of one of them, they become more or less refractory to all other antigens at the same time. True, the solidity of this nonspecific antianaphylaxis in the *in vivo* experiments, was claimed by many investigators to be less marked against heterologous antigens[41,24]; also *in vitro*, Dale observed "that uterus of a guinea pig sensitized against two different antigens and then desensitized completely to one of them, loses a certain amount of sensitiveness to the other."[15] However, other investigators found that desensitization to all antigens was approximately equal in solidity.[4,5,21,36*] It is clear that under these circumstances, the refractoriness must be due to some mechanism other than saturation of specific antibodies in the tissues.

It is clear that refractoriness of these animals to antigens other than the one with which these animals have been "desensitized" cannot possibly be due to the specific saturation of sessile antibodies.

The notion that sessile or fixed antibodies play a decisive role in the mechanism of anaphylaxis has its origin in early experiments in which it was found that a certain amount of time (several hours) was necessary

*Some unpublished experiments carried on in my laboratory many years ago, have indicated that this discrepancy could be eliminated if animals used for such experiments were uniformly sensitized (that is, if instead of active sensitization one employed a passive sensitization with a uniform amount of antibody), if the antibody were derived from homologous animals, and if the antigens used for test injections were carefully standardized before the test and the doses injected were equivalent in their anaphylactogenic potency.

before the passively sensitized animal was able to react to a test injection of the antigen.[40]† In spite of the fact that in the rabbit, dog and mouse[34,35] passive anaphylaxis could regularly be elicited by injection of antigen immediately after introduction of a sensitizing serum (as well as by an injection of a mixture of antigen and antibody into normal animals, including guinea pigs)[25], the relative irregularity in the results of such experiments when performed in guinea pigs (in whom allowing for an incubation period between passive sensitization and test injection, seems to contribute to a greater regularity of results), was responsible for the postulate that incubation period for the "fixation" of antibody was essential.

However, more recently, even some of those investigators who previously insisted on the particular significance of the incubation period, (which was necessary for the fixation of antibody by the tissues in guinea pigs), have found that passive anaphylaxis can be produced in guinea pigs without allowing any "period of incubation."[16,54] Moreover, in the experiments performed *in vitro* with red blood cells[17,28,29], and with strips of uterus and small intestine[32], it was found that union between antigen and antibody occurring in the environment into which normal red cells or tissues, respectively, are introduced, causes an injury resulting in the liberation of histamine from these normal cells without incubation period.

Such considerations as these suggest that, while actively sensitized animals undoubtedly possess fixed tissue antibody, the assumption that in passively sensitized animals the tissue becomes reactive *only* when and if antibody becomes fixed to the tissues is not well founded. Moreover, these findings make very doubtful the validity of the hypothesis that "desensitization" is accomplished by saturation of fixed antibodies.*

This discussion was intended to make clear that the term "desensitization" is a misnomer. The temporary relative refractoriness of a hypersensitive animal, achieved through the administration of a suitably chosen dose (either single or multiple) of antigen does not decrease the basic specific sensitivity of the animal. In fact, though not immediately, it tends to increase it. Moreover, this temporary refractoriness is secured neither by the interposition of circulating antibody between the antigen and sensitive cells, nor by the saturation of fixed antibodies. Apparently the refractoriness is determined by some other mechanism entirely outside of antibody balance.

That the state of refractoriness must rest on a different mechanism than *true* desensitization is evident also from other considerations.

Mention has already been made of the fact that the refractoriness resulting from exposure of multiply sensitized animals to one of the respec-

†Doerr and Russ: Ztschr. f. Immunitäts., Orig., 3:181, 1909; Manwaring: Ztschr. f. Immunitäts., Orig., 8:1, 1910-11; and Weil, J.: Med. Res. 28:85, 1913.

*The latter conclusion, however, cannot be stated more definitely until such time when it is shown experimentally that normal uterine strips exposed to such mixtures of antigen and antibody become refractory to a subsequent exposure to similar mixtures.

tive antigens will render these animals (or isolated tissue)[15] relatively refractory to any other antigen to which they happen to be simultaneously sensitized. Thus, while in this instance, the process of establishing the refractory state is specific, that is, it depends upon the introduction of a particular anitgen to which specific sensitivity exists, the resulting effect of the refractory state is *not* specific—the animal exhibits diminished reactivity to the effects of introduction, not only of this particular antigen, but also of any other antigen to which the corresponding antibody is also present.

On the other hand, occasionally a diminution in reactivity of a sensitized animal to the injurious concomitant effects of antigen-antibody union (refractoriness to anaphylaxis) has been observed to occur spontaneously (without any "desensitization"), as for instance in allergic individuals suffering or recovering from measles, scarlet fever, cancer, or during pregnancy, etc. Furthermore, a state functionally very closely resembling the refractory or antianaphylactic state may be produced at will artificially by a parenteral introduction of a great variety of substances (other than specific antigens). As a group, these substances (antigenic or nonantigenic *per se*, organic or inorganic) are characterized by the fact that they are capable (by virtue of their inherent toxicity) of eliciting anaphylaxis-like ("anaphylactoid") reactions when injected into normal animals.

The pathological changes found as result of injection of anaphylactoid agents are strikingly similar to those found in anaphylaxis and more recently (particularly in the case of peptone[19] and trypsin)[42,43,44,45], it was found that both these changes and the symptoms observed in anaphylactoid reactions are due to the liberation of histamine, the mechanism which is strongly suspected of being responsible for similar changes in true anaphylaxis.

However, while this resemblance is very strong, the two phenomena differ from each other in some minor respects due unquestionably to the secondary effects of the individual properties of the different anaphylactoid agents employed, the effects which are superimposed over the basic reactions. For this reason, while admitting the major resemblances, some of the investigators are cautious in drawing conclusions as to their significance. Thus, for instance, Topley[47], in discussing the resemblance between "anaphylactic" and "anaphylactoid" reactions, states:

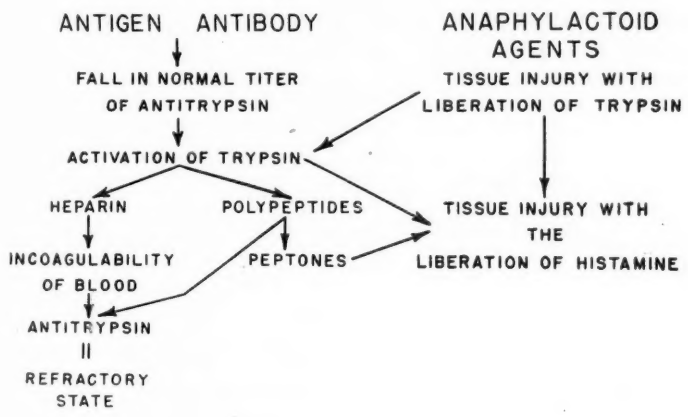
". . . The resemblances are certainly striking and it seems that common factors are involved; but there are certain differences."

". . . The resemblance is not due to any similarity in the underlying mechanism, but to the fact that, in each case, cellular injury is followed by the liberation of histamine."[50]

On the other hand, to many investigators, these resemblances seem very significant. Thus Hanzlick[26], on the basis of his extensive study of anaphylactoid reactions, came to the conclusion that the mechanism involved

in these reactions is basically related to that which operates in true anaphylaxis.

About thirty years ago, before the role of histamine in anaphylaxis was definitely suggested, we found that the products of interaction of antibody with antigen *in vitro* resulted in the activation of serum trypsin



and subsequent autodigestion of serum.[6] Either trypsin itself, or the products of its action (both of which are present in the mixture), when injected into animals produced symptoms indistinguishable from anaphylaxis.[8] We found also that serum properly absorbed with kaolin or starch *in vitro* has similarly caused activation of serum ferments[12], with subsequent autodigestion of the serum, which became capable of producing anaphylaxis-like symptoms when injected intravenously, especially in homologous animals.[9,10]

These early findings receive support and are considerably more meaningful now that it has been found that either reaction of antigen-antibody as such, or trypsin (or some products of its activity) are capable of setting free performed histamine from the erythrocytes[28,29], skin[30], or smooth muscle[42] *in vitro*, and thus presumably also *in vivo*.

Furthermore, some of these early findings have also suggested what I think is the true mechanism responsible for the refractory state.

As the serum-tryptase becomes activated (through physico-chemical changes initiated by the antigen-antibody union)[9]*, the products of its digestive activity have a tendency to delay or even arrest further activity of the enzyme.[11] They exert so-called "antitryptic" activity.[1] Some of these products have been identified more recently as polypeptides.[30] Undoubtedly other constituents of the serum (as, for instance, normal serum albumin[7], unsaturated fatty acids†) and products appearing during

*See also Dale, H. H.: Lancet, 1:1285, 1929.
†Schwartz: Wien. Klin. Wchnschr., 22:1151, 1909; Bauer, J. Z.: Zeitschr. f. Immunitäts., 5:196, 1910; Jobbling, J. W., and Petersen, W.: Jour. Exp. Med., 19:239, 251 and 459, 1914.

the reaction *in vivo* (for instance, heparin)[18,33], are also capable of inhibiting the activity of trypsin. Some of these substances, spoken of collectively as "antitrypsin" are not dialysable; thus, once a high antitrypsin content is established, it may persist for some time. We have shown that irrespective of the method by which a rise in antitrypsin was produced—whether specifically (as a result of antigen-antibody reaction)[12], or non-specifically by (injection of anaphylactoid agents)[13], the animals remain refractory to anaphylaxis so long as the antitryptic titer remains elevated.[11,14,46]

The refractory state, therefore, does not involve the antibody balance but seems to be due to the fact that the environment (in the general circulation or at the cell surface) is so changed by the presence of trypsin-inhibitors (antitrypsin) that any subsequent liberation of trypsin finds the medium unsuitable for its activity, and hence no further liberation of histamine (due to the tissue injury caused by trypsin itself, or by some of the toxic products of its activity) takes place.

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HISTAMINE TOLERANCE

By R. Katzenstein, M.D.

(Condensed from the *Yale Journal of Biology and Medicine*, March 16, 1944, pp. 325-331).

Corroborative evidence of acquired tolerance to histamine by the dog is presented. Additional studies of canines with such acquired tolerance were made to ascertain any possible resultant structural changes and its functional manifestations. These observations included high altitude reactions and changes in the ketosteroid content of the urine. A brief review of the increased tolerance to histamine by guinea pigs and dogs as shown by Horton and his associates and Karady is presented. This type of functional disturbance is distinguished from tachyphylaxis: a rapid adaptation to small doses of histamine, observed by Karady and by Eichler and Killian. The latter were able to inject 150 mg. of histamine into rabbits without producing their death if done within a half hour after a preliminary small injection. The structural changes following histamine death when the latter occurs sufficiently late (twenty-four hours) vary from necrobiosis of cerebral or cerebellar ganglion cells to cerebral infarcts without vascular occlusion. Other lesions observed due to lack of tolerance are peptic ulcer of the stomach and duodenum, adrenal cortex hemorrhage and marked passive congestion of the viscera, particularly the liver.

Experimental Study

An initial dose of 1 mg. of histamine for a dog of 10± kg. slowly increased by successive injections over a protracted period protected the animal from death when a single injection of 50 mg. was made. In seven of eight dogs subjected to this procedure only minor anatomical changes were demonstrable. The author noted that the increase in the amount of histamine tolerated becomes limited with successive doses and that structural changes are more likely to result when symptoms of the more severe type occur while developing tolerance.

A second group of thirteen animals received single or repeated doses of sufficient amounts of histamine to cause severe and protracted symptoms. There was a marked contrast in the results obtained compared to those receiving histamine tolerance doses. The histamine shocked group manifested extensive lesions of the heart, central nervous system and alimentary canal. The most constant changes occurred in the heart with yellow mottling seen from the epicardial and endocardial surfaces as well as the cut surfaces of the myocardium, due to focal accumulations of fat with their necrosis and peripheral collections of mononuclear cells. Early lesions of the alimentary canal were edema, congestion and hemorrhage of the mucosa and submucosa. Later changes are followed by focal necrosis with ulceration. Focal hyaline necrosis of the spleen was most frequent in this organ, although anemic and hemorrhagic infarcts may be observed.

Tachyphylaxis was also observed when recording the blood pressure following small doses of histamine. There were no changes of symptoms and the animals quickly recovered from a single dose of 6 mg. per kg. which would prove fatal in ten minutes for untreated dogs.

Tachyphylaxis also did not cause hypertrophy of the adrenal cortex as it does in the rat.

Two groups of animals were subjected to varying high altitudes, up to 41,000 feet above sea level, in checking both normal controls and histamine-tolerant dogs. Dr. L. F. Nims, who aided in the high altitude experiments, expressed the criteria of the reaction of the dogs to the high ceiling by indicating that the respiration rate increased proportionately to the decreasing atmospheric pressure. There was no appreciable difference observed in the "respiratory ceiling" or the urinary 17 ketosteroid content of the histamine tolerant and the control animals.

MOLD FUNGI IN THE ETIOLOGY OF RESPIRATORY ALLERGIC DISEASES

III. Immunological Studies with Mold Extracts

1. Preparation of Experimental Extracts

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CLINICAL experiences with extracts prepared by The Association of Allergists for Mycological Investigations have been confusing. While in many instances diagnostic reactions have been obtained with high dilutions, most of us have become skeptical lest the use of higher concentrations has resulted in an unwarranted number of falsely positive tests. Such nonspecific reactions possibly could incriminate certain of our extracts as being both impotent and irritative. In 1942, Browning[1] reported on the mathematical evaluation of our extracts and confirmed the impression that this irritative type of reaction is entirely too frequent with many of them.

At the Little Rock meeting of our group in 1942, it was, therefore, decided that more effort be devoted to a study of various methods of preparing mold extracts with the object of obtaining more efficient and less irritating antigens.

Our routine method of extract preparation has been described elsewhere.[3] Briefly, the pellicles are grown to maturity on a standard malt extract broth (Difco), harvested, washed with several changes of normal saline to remove whatever irritants might be contained in the culture medium, and dried to constant weight at 40° C. Some early extractions were made in buffered saline but a modified Hollister-Stier extracting fluid was substituted in 1940, in the hope that more permanent antigens could be obtained. Extracts prepared in this manner on a weight-volume ratio of 1:20 are still in use, and in the following remarks will be referred to as extracts prepared by the usual method. The Hollister-Stier solution has been modified by the addition of "Merthiolate" (Lilly), to a concentration of 1:10,000 by weight.

If we are to condemn our extracts as irritative or impotent, let us consider the possible sources of error in their preparation. In the first place we have subjected these wet mold pellicles to a slow-drying process and have proceeded from there just as we work with such better known antigens as pollens. About 1939, Nelson suggested that in slow drying, the pellicles might undergo an irreversible hardening process somewhat

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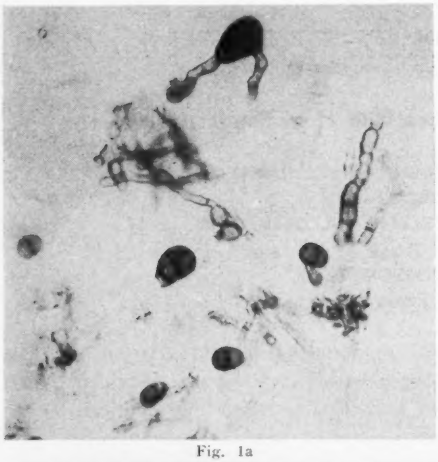

Fig. 1a

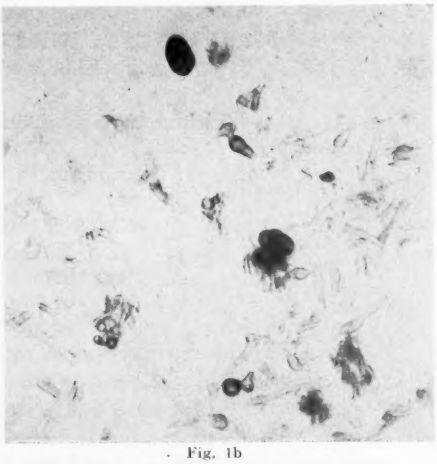

Fig. 1b

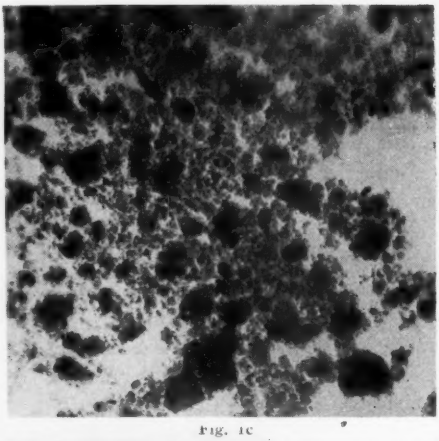

Fig. 1c

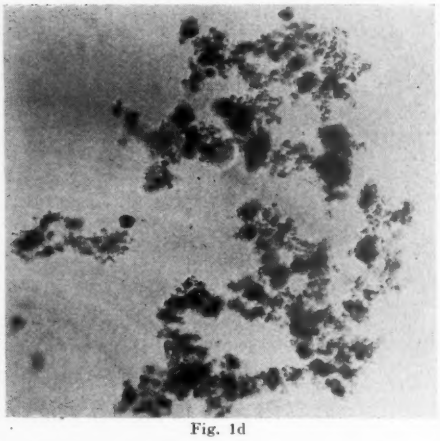

Fig. 1d

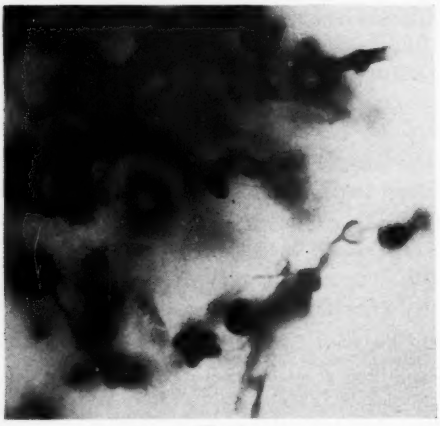

Fig. 1e

Fig. 1. Photomicrographs of *Alternaria tenuis* Nees BC-17, prepared for extraction by various methods.

(a) Dried by lyophilization. (b) Dried by lyophilization and defatted. (c) Dried by lyophilization, defatted and ground. (d) Dried by lyophilization and ground. (e) Dried to constant weight at 40°C., according to the usual method.

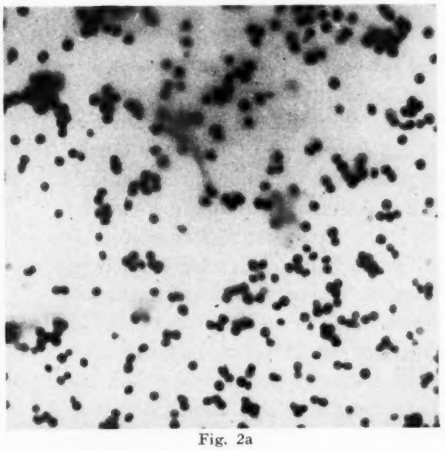

Fig. 2a

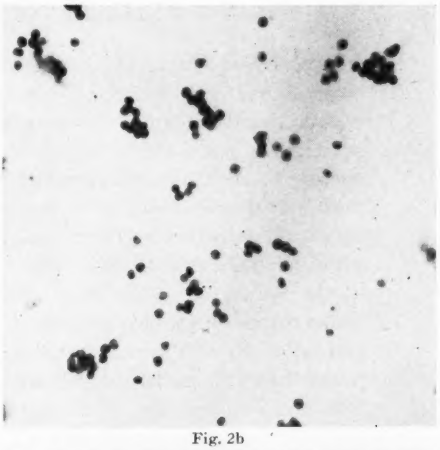

Fig. 2b

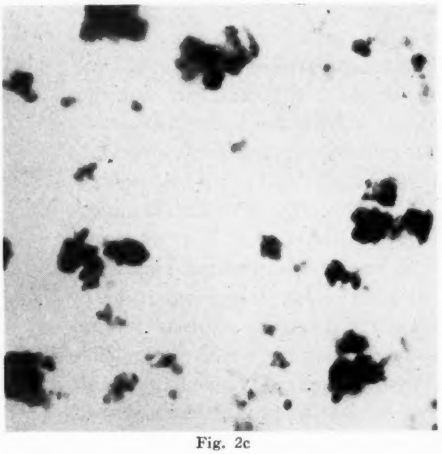

Fig. 2c

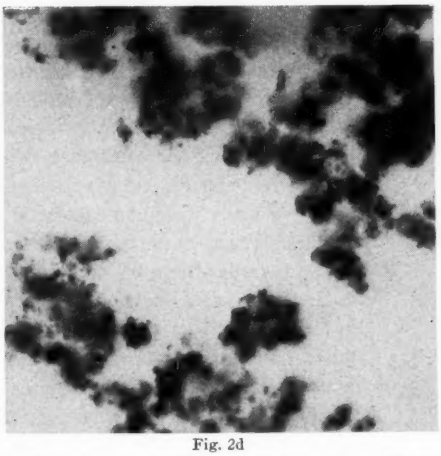

Fig. 2d

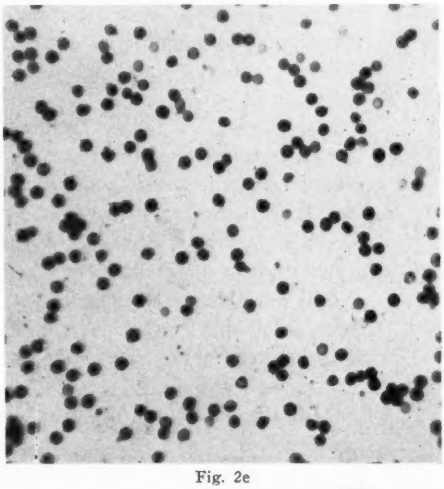

Fig. 2e

Fig. 2. Photomicrographs of *Aspergillus niger* BC-70, prepared for extraction by various methods.

(a) Dried by lyophilization. (b) Dried by lyophilization and defatted. (c) Dried by lyophilization, defatted and ground. (d) Dried by lyophilization and ground. (e) Dried to constant weight at 40°C., according to the usual method.

comparable to coagulation, whereby the active principle might either become altered or at least not again freely soluble in water.

Again some controversy has arisen regarding just wherein the active principle is contained in molds. While no definite proof has been forthcoming to show that allergens in the spores and mycelium are identical, most workers feel that more active material actually is contained in the spores than in the mycelium[2] and, therefore, have assumed that if they could produce more spores they could make better extracts. Sometime in the spring of 1942, Dr. G. W. Goldsmith, of the Department of Botany and Bacteriology of the University of Texas, mentioned to us and Wittich, that some previous work on mold spores had revealed intracellular molecular concentrations sometimes approximating 275 atmospheres of pressure. He suggested, furthermore, that the higher concentrations would be expected in smaller, more spherical spores, such as those of Penicillium and Aspergillus, and conversely that larger spores like Alternaria, Helminthosporium and other members of the Dematiaceous group could have less intracellular concentration. From a practical standpoint, this suggests that it would be difficult or even impossible to extract active principles from within the spores, especially those of the higher molecular concentrations, provided the spore walls actually were tough enough to present an obstacle, and provided, further, the spore walls were not ruptured prior to or during the process of extracting. From these considerations it occurred to us that we might have an explanation of why we have been able to produce apparently more active extracts from the Dematiaceous molds than from some of the others, such as species of Aspergillus and Penicillium. In a few instances one of us (Prince) has observed apparently definite, reliable skin tests from extracts of certain species of Aspergillus and Penicillium, but such cases are certainly not nearly as frequent as one would expect to encounter, considering the widespread distribution of these mold forms. We have often wondered if the few significant reactions obtained with Aspergillus and Penicillium could not have resulted from antigenic material extracted from the mycelium rather than from the spores.

The matter of defatting antigenic material before extraction has given rise to some argument. However, there seems to be a rather general consensus of opinion that pollens yield better extracts if previously subjected to removal of waxes and lipoids by fat solvents. We have wondered, therefore, just what significance defatting would have in the preparation of mold extracts. Except for a few experimental extracts which were not systematically studied, our routine pellicles have not been defatted. Furthermore, it is a known fact that mold material often contains a high percentage of lipoids, up to approximately 40 per cent in some species.

With the object of investigating these three major considerations, a

series of experimental extracts was begun in the spring of 1943, using molds planted in malt extract broth in November, 1942. The flasks containing the mature pellicles with maximum sporulation were shaken vigorously to dislodge the pellicles from the surface of the broth, and the broth was decanted through filter paper. The pellicles were washed with 20 successive changes of normal saline in amounts equivalent to approximately the volume of the original broth. With these washings each flask was shaken vigorously and the saline allowed to stand on the pellicle a few minutes. To convenient portions of the broth and of the fourth, tenth, fifteenth, and twentieth washings was added 1:10,000 "Merthiolate" and these portions saved for future study. After the last washing the pellicles were cut in thin strips, frozen rapidly between cakes of dry ice and dried by lyophilization. All containers were sealed immediately after drying to prevent reabsorption of moisture from the air.

The dried pellicles were then divided into four portions. The first portion was not further treated. The second portion was defatted with anhydrous ethyl ether in Soxhlet equipment protected by calcium chloride to prevent absorption of moisture from the air. The third portion was similarly defatted and ground in a porcelain ball mill with smooth flint pebbles for several days. The fourth portion was placed immediately into the ball mill without preliminary defatting. All transfers of the dried material from one container to another were made in a dehydrator cabinet. This precaution was instituted after previous work had revealed that lyophilized material is quite hygroscopic and rapidly takes up considerable moisture in ordinary air. A fifth portion of pellicle was prepared by the usual method of slow drying. From these variously treated pellicles, five extracts were prepared simultaneously in Hollister-Stier fluid containing 1:10,000 "Merthiolate" on a weight-volume ratio of 1:20 and sent out to the members of our group in October, 1943. The extracts prepared by the first four methods were designated by number only, but the members were informed that extract five was prepared by the usual technique.

To recapitulate, these extracts were prepared as follows:

1. Dried only by lyophilization
2. Dried by lyophilization, defatted
3. Dried by lyophilization, defatted, ground
4. Dried by lyophilization, ground
5. Usual method (slow drying)

Two different molds were selected for these various methods of extract preparation—the two being *Alternaria tenuis*, Nees, and *Aspergillus niger*, Thom and Church. Alternaria was selected because so far it seems to be the most prevalent mold with which most of us are familiar, and because it seems to be a frequent troublemaker among the molds. Aspergillus, on

the other hand, was selected for just the opposite reason; it has not been associated frequently with respiratory allergy, even though it is widely distributed. The members were asked to make serial dilutions from 1:100 to at least 1:100,000 and to perform testing both on known Alternaria-sensitive and possibly Aspergillus-sensitive patients, and on patients not considered sensitive to either of these molds.

The photomicrographs of the variously treated mold material (Figs. 1 and 2) reveal that spores and mycelium of both the Alternaria and the Aspergillus were very thoroughly broken up in the ball milling process. We were quite surprised to note that this was true regardless of whether the pellicles had previously been defatted; we had anticipated difficulty in grinding the undefatted material. A very definitely darker color was observed in both the Alternaria and Aspergillus extracts prepared by Method 3, in which the pellicles were dried by lyophilization, defatted and ground.

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REACTIONS TO PARENTERAL FLUID ADMINISTRATION. Strumia et al: Ann. Int. Med., 19:718, (Nov.) 1943.

The various causes for reactions are discussed. Classification includes: (1) Causative agents inherent in fluid alone (pyrogenic, nitritoid, embolic and mechanical). (2) Inherent qualities of fluid combined with condition of patient. Included here are hemolytic and allergic—latter reactions attributed to substances, alimentary in origin, contained in whole blood, plasma or serum to which the recipient is sensitive. Localized urticaria is usually present, but may be generalized and associated with angioneurotic edema and rise in temperature. Asthma is occasionally seen. The danger of edema of the glottis must be considered, since it occurs in 0.3 to 1 per cent of all transfusions. It should be insisted that the donor be fasting. Reactions respond well to epinephrine. True anaphylactic reactions are rare. (3) Conditions inherent in recipient alone (hyperhemolysis, liver disease, hypoproteinemia, cardiac insufficiency). (4) Temperature, air emboli, free hemoglobin, etc.

L. J. H.

MANAGEMENT OF PAROXYSMAL TACHYCARDIA INCLUDING THE USE OF MECHOLYL. Morgan, P. W.: Ann. Int. Med., 19:780, (Nov.) 1943.

Mecholyl (acetyl-beta-methylcholine) causes a bronchial spasm abolished by epinephrine or by atropine. It is contraindicated in the treatment of paroxysmal tachycardia for those patients who also have bronchial asthma.

L. J. H.

MOLD FUNGI IN THE ETIOLOGY OF RESPIRATORY ALLERGIC DISEASES

III. Immunological Studies with Mold Extracts

2. Skin Tests with Experimental Extracts

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SKIN testing with extracts prepared by the various methods for this study has been performed on patients sensitive to Alternaria and on individuals not considered to be sensitive to Alternaria. While several testing records are complete with intradermal titrations in strengths from 1:100,000 to 1:100 including scratch testing using 1:20 concentration for all five extracts, intradermal tests with 1:1,000 dilutions have been most frequently performed and will of necessity form the basis of this report. In some instances definitely positive reactions have been obtained with 1:10,000 or 1:100,000 dilutions but have not been carried out in higher concentrations. On the other hand, many testing records revealed that observations only in concentrations of 1:100 had been made, or that the 1:1,000 strength had not been used for both Alternaria and Aspergillus, or the records were in some other detail incomplete and could not therefore be incorporated into this report.

Table I shows the results of intradermal testing on fifteen patients with respiratory allergy in whom sensitization to Alternaria was considered a major, although not necessarily the only, etiological factor. Only in Cases 7, 8 and 9 were definite reactions consistently obtained with *Aspergillus niger*. The patient in Case 7 was considered definitely sensitive to *Aspergillus niger* and patient 8 was considered probably sensitive to *Aspergillus niger*. Both patients in Cases 7 and 8 had *Aspergillus niger* included in their therapeutic mixtures. Patient 9 had not been considered sensitive to *Aspergillus niger* in spite of the fact that he reacted fairly consistently with the various experimental extracts. No differences can be observed which might be attributed to any variation in preparation of the experimental extracts, nor as distinguishing the experimental from our ordinary type of extract. The latter statement is further borne out by the test records of "other" extracts which are in all instances routine preparations previously supplied the various investigators; in case 4 the extract was made from dried pellicles of Alternaria prepared in our routine fashion and supplied to the investigator for his own extraction; in Cases 9, 13, 14 and 15 the testing antigen was a pool of our Alternaria extracts; in Cases 7 and 8 the extracts of *Alternaria tenuis* and *Aspergillus niger* were

Presented before the Association of Allergists for Mycological Investigations, St. Louis, Missouri, January 21, 1944.

TABLE I. INTRADERMAL SKIN TESTING WITH EXPERIMENTAL EXTRACTS 1/1000 IN PATIENTS IN WHOM ALTERNARIA IS A MAJOR ALLERGEN.

	ALTERNARIA TENUIS						*ASPERGILLUS NIGER*											
Method	1	2	3	4	5	Other	1	2	3	4	5	Other	INVESTIGATORS	Winter	Spring	Summer	Fall	OTHER IMPORTANT ALLERGENS
Case 1	++	+	?	++	++		−	−	−	−	−		KDF Asthma Hay fever		x	x	x	Ragweed
2	++	+++	+	+	++		−	−	−	−	−		KDF Asthma Hay fever	x	x x	x x	x x	Ragweed, dander
3	+++	−	+	+	?		−	−	++	++	−		KDF Asthma		x	x	x	
4	+++	++	+++	++++	+++	+++	−	−	−	−	−		KDF Asthma Hay fever		x	x	x	
5	+++	++	+	++	++		−	−	−	−	−		KDF Asthma	x	x	x	x	Dust, ragweed, grass, wheat
6	++	?	+	++	+		?	?	+	?	?		KDF Asthma	x	x	x	x	Ragweed, grassdust, dander
7	++++	++++	+++	++++	+++	++	++	+++	++++	++++	+++	++	FWW Asthma	x	x	x	X	Pollens
8	+++	++	++	+++	+++	+++	++	++	+++	+++	+++	+++	FWW Asthma Hay fever	x	x	x	X	Pollens, foods
9	++	++	+	++	++	+++	++	++	+	+++	+		HEP Asthma Hay fever	x	X	x	X	Ragweed, grass, dust
10	−	++	+	+	+		−	++	−	+	−		EDS Asthma	x	x	x	x	Pollens, dust
11	?	+	+++	++	+++		?	−	?	−	+		HEP Asthma Hay fever	x	x	x	x	Ragweed, dander dust, food
12	+	++	+	+++	++		+	+	−	?	+		HEP Hay fever	x	X	x	X	Ragweed, dust, grass
13	++	++	++	+++	++	+++	+	?	+	+	?		HEP Asthma	x	X	x	x	Dust, grass
14	+	++	+	+++	++	++	+	+	+	?	−		HEP Hay fever	x	X	x	X	Dust, ragweed, hickory
15	++	+++	++++	++++	+++	+++	−	?	?	?	?		HEP Asthma Hay fever	x	x	x	X	Ragweed, dust, grass

Capital X indicates that in the season specified, the patient's symptoms are increased.

TABLE II. INTRADERMAL SKIN TESTING WITH EXPERIMENTAL EXTRACTS 1/1000 IN INDIVIDUALS NOT CONSIDERED PRIMARILY SENSITIVE TO ALTERNARIA.

	ALTERNARIA TENUIS						*ASPERGILLUS NIGER*											
Method	1	2	3	4	5	Other	1	2	3	4	5	Other	INVESTIGATORS	Winter	Spring	Summer	Fall	OTHER IMPORTANT ALLERGENS
Case 1	−	−	−	−	−		−	−	−	−	−		HEP Asthma	x	x	x	x	Asp. terreus, Asp. nidulans, chronic bronchitis
2	+	++	+++	++	−	+++	+	?	−	++	+	+	PTP Asthma Hay fever	x x	x x	x x	X x	Dust, ragweed-marsh elder, danders

3	+++	++	+++	+++	++	+	?	+	+	+	−		HEP	Asthma	x	x	x	x	Dust, ragweed
4	?	+	−	+	?	+	−	+	−	?	−	?	PTP	Asthma Hay fever	x x	x X	x X	x X	Dust, ragweed-marsh elder, grass
5	−	−	−	−	−	?	?	?	−	−	−	−	PTP	Hay fever	x	x	x	X	Dust, ragweed-marsh elder, feathers
6	−	−	?	?	−	−	−	−	−	−	−	−	PTP	Asthma	x				Dust, foods
7	−	?	?	?	?	?	−	?	−	−	−	−	PTP	Asthma	x	x	x	x	Dust, ragweed-marsh elder
8	?	+	−	−	?	?	−	−	−	−	−	−	PTP	Asthma Hay fever	x x	x	x	x	Dust
9	+	+	−	?	−		−	−	−	−	−		HEP	Hay fever	x	x	x	x	Dust
10	−	+	+	+	+		+	?	−	+	−		HEP	Asthma	x	x	x	x	Dust, bronchitis
11	−	?	−	?	−	?	−	−	−	−	−	−	PTP	Asthma	x	x	x	x	Dust, bronchitis, emphysema
12	−	−	−	−	−	−	+	−	−	+	+	−	FWW	Asthma Dermographia	x	x	x	x	Dust, foods, smuts
13	−	+	−	−	−	−	−	−	−	−	++	+	FWW	Hay fever	x	x	x	x	Dust, foods
14	−	−	−	−	−	−	−	−	−	−	−	−	FWW	Asthma Eczema	x	x	x	x	Dust, foods
15	−	?	+	+++	+		−	−	++	+++	+		JAM	Asthma, hay fever, eczema	x	x	x	x	Dust, ragweed, grass, danders
16	−	−	−	−	−		−	−	−	−	−		JAM	Asthma Hay fever	x	x	x	X	Ragweed, dust, orris root
17	−	−	−	−	−	−	−	−	−	−	−	−	FWW	Hay fever				x	Ragweed
18	−	−	+	+	+		−	−	−	−	−		JHB	Asthma Hay fever	x	x	x	x x	Dust, ragweed
19	+	+	−	−	?	+	−	?	−	−	−	−	PTP	Asthma	x	x	x	x	Dust
20	+	++	−	−	?	+	−	−	−	−	−	−	PTP	Asthma Hay fever	x x	x x	x x	x X	Dust, ragweed-marsh elder, orris root grass
21	−	−	−	−	−	−	?	−	−	−	−	−	FWW	Nonallergic					
22	−	−	++	−	−	+	−	?	?	−	−	+	FWW	Nonallergic					
23	−	−	−	−	−	−	−	−	−	−	−	−	FWW	Nonallergic					
24	−	−	−	−	−	−	−	−	−	−	−	−	FWW	Nonallergic					

Capital X indicates that in the season specified, the patient's symptoms are increased.

routine distributions at an earlier date. Finally, differences in the reacting abilities of the various patients are maintained throughout most of the tests, irrespective of the modification of the particular extract.

Table II shows the results of intradermal testing with 1:1,000 dilutions in twenty-four patients in whom Alternaria sensitization was not considered of primary importance. The first twenty patients have respiratory allergies, while the last four are non-allergic.

A few interesting facts regarding the first three patients in this series might be mentioned. In Case 1 the patient did not react by scratch or by intradermal tests in all concentrations to either *Alternaria tenuis* or *Aspergillus niger*. However, in previous routine testing he had reacted to nothing except *Aspergillus nidulans* and *A. terreus*, in dilutions of 1:1,000, and therapy with these molds produced a good result. In Case 2 the patient had a very reactive skin which responded to practically everything with which he was tested, especially to cow hair, house dust and feathers. In Case 3 the patient reacted only to dust and ragweed in addition to Alternaria, but since he has been perfectly relieved on a dust and ragweed regimen without the employment of molds in his therapy, the mold reactions are not taken to indicate major sensitizations in his present environment. In Case 4 Alternaria was considered by the investigator to be of secondary clinical importance. In Cases 5 through ten, inclusive, molds were considered by the investigators to be of questionable importance, while in the remaining ten of the allergic patients they were considered entirely unimportant.

As in the first group, there are no outstanding differences in this series that might be attributed to any variations in the preparation of the experimental extracts.

CONCLUSIONS

The observations herein presented as well as other unpublished data do not indicate any differences in any of the experimental extracts of *Alternaria tenuis* and *Aspergillus niger* that could be attributed to variations in the technique of preparation. Furthermore, the experimental extracts are about as active as extracts of the same materials prepared by our usual methods.

A CASE OF ASPIRIN POISONING—M.D. Charters. Brit. M. J., 1:10, (Jan.) 1944.

Case report of a patient who had taken 750 gr. aspirin in a suicidal attempt. Toxic signs began to appear about fourteen hours after taking the drug. Pyrexia was mild. Hyperpnea was marked and probably due to acidosis, resulting from either direct action of the acid radical of the drug or from the disordered metabolism. Disorientation was the only mental reaction. Toxic hepatitis resulted. Recovery of the patient is reported, with the excretion of the drug continuing over a period of at least four days.

L. J. H.

MOLD FUNGI IN THE ETIOLOGY OF RESPIRATORY ALLERGIC DISEASES

III. Immunological Studies with Mold Extracts

3. Failure to Find Histamine-Like Substances in the Washings and Extracts of Molds Used for Skin Testing

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ALTHOUGH the significance of molds as allergic excitants of hay fever and asthma has been well established, the value of skin tests in determining mold antigens has often been questioned. Browning[2] has recently summarized results on a large number of extracts made available to members of the Association of Allergists for Mycological Investigation. In a series of thirty-eight extracts tested intracutaneously, he reported that 37 per cent were irritants and had no clinical value. Prince[14], likewise observing marked reactions with certain of these extracts, has also expressed the opinion that some appear to be of the irritative class. On the other hand, Harris[12], using different extracts and testing intracutaneously, found an incidence of 38.5 per cent positive Alternaria reactions in 130 patients with seasonal hay fever and asthma; his observations were reported to be statistically significant and to indicate that the extracts were nonirritative. Similar observations have been reported by Feinberg[8], who also expressed much faith in skin testing, particularly on the scratch-test basis and with concentrated extracts or dry powder.

Realizing that the technique of preparation might be responsible for the irritating properties of some mold extracts, it was decided to assay the broth and washings of several fungi, together with their extracts, for histamine-like substances. When one recalls that histamine is widely distributed in the tissues of plants, as well as animals, and that it was first identified by Barger and Dale[1] in a fungus, *Claviceps purpurea* (ergot), its presence in mold extracts should not be surprising. Furthermore, the various broths on which the experimental molds are grown usually contain peptone, which, when contaminated by certain bacteria, is converted to histamine, as was shown some years ago by Hanke and Koessler[11], and more recently by Roske.[17]

SOURCE OF MATERIAL, METHODS OF GROWING, AND PREPARATION OF EXTRACTS

Two molds, *Alternaria tenuis,* and *Aspergillus niger,* were selected as suitable materials for the tests reported here. Various surveys by Feinberg and Little[9], Durham[6], and Morrow, Lowe and Prince[13], have indicat-

Presented before the Association of Allergists for Mycological Investigations, St. Louis, Missouri, January 21, 1944.

Fig. 1. A typical record showing the ineffectiveness of washings of *Aspergillus niger* and the effectiveness of minute amounts of histamine on the guinea-pig intestine. (In this and subsequent figures "1st washing" is used synonymously with "culture broth").

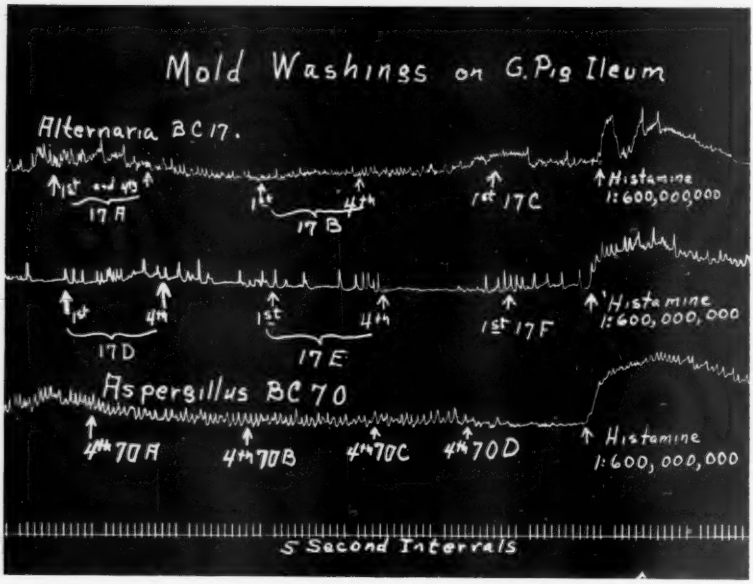

Fig. 2. Group testing of Alternaria BC-17 *(A. tennis)* and Aspergillus BC-70 *(A. niger)*. The letters A, B, C, D, et cetera, after numbers 17 and 70 refer to different preparations of the same mold.

ed that Alternaria and Aspergillus are widely distributed and are among the most frequent air-borne molds causing inhalant allergy. Feinberg[7] found that Alternaria gives positive skin reactions more frequently than other fungi, with yeast second and Aspergillus a close third. With the Alternaria group of extracts made available to members of the Association of Allergists for Mycological Investigations, Browning[2] found 29 per cent positive skin reactions in 979 patients; Chobot, Dundy and Schaffer[3], using other extracts, observed 28.2 per cent positive reactions in 117 cases. With extracts of the Aspergillus group, Browning reported 23.7

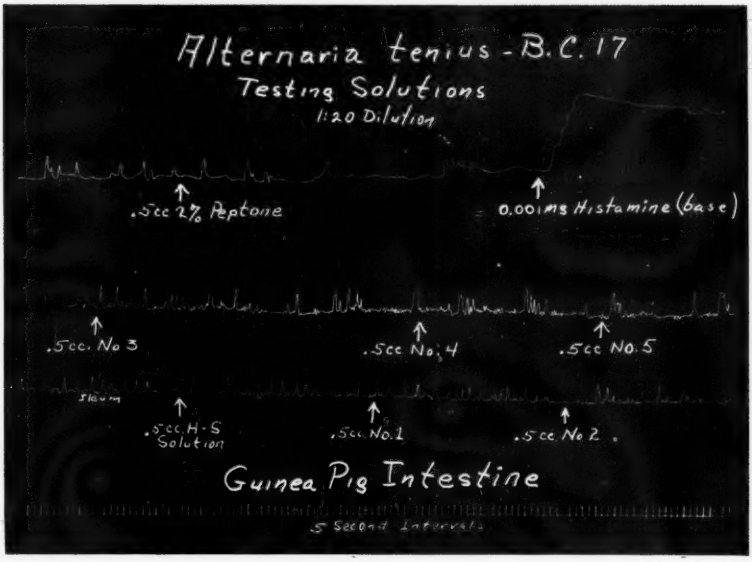

Fig. 3. The ineffectiveness of five mold extracts of *Alternaria tenuis* prepared by different methods of extraction. Record begins at lower left. "H-S" refers to the Hollister-Stier extraction fluid.

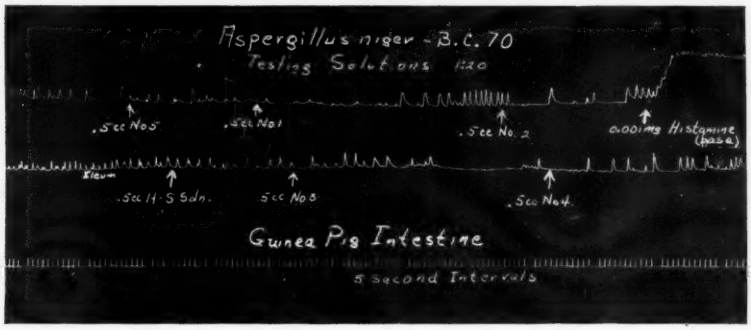

Fig. 4. The ineffectiveness of five mold extracts of *Aspergillus niger* prepared by different methods of extraction. Record begins at lower left. "H-S," Hollister-Stier extraction fluid.

per cent positive reactions in 1,092 patients; Chobot and his associates reported 16.1 per cent in 117 cases.

The source, distribution and identification of these molds have been described by Morrow, Lowe and Prince.[15] The general procedures employed in growing, harvesting and preparing extracts of these molds have been described by Prince and Morrow.[15] In the preparation of the present extracts, five different methods were used; these have been discussed in a preceding paper.

Testing for Histamine by the Guinea-pig Method.—The methods employed were essentially those originally described by Schultz[18] and modified by Dale.[5] Each solution was tested on an isolated uterine horn or a segment of intestine (ileum) obtained from a half-grown guinea pig. The strips of smooth muscle were immersed in oxygenated Ringer's solution adjusted for a pH of 7.4 and maintained at a temperature of 30° C. until used. When testing, the strips were suspended one at a time in 150 c.c.

TABLE I. HISTAMINE SENSITIVITY OF TWENTY-SEVEN NORMAL SUBJECTS

Response to 0.02 c.c. Intracutaneously		
Concentration	Number Positive Reactions	Per Cent Positive Reactions
1:50,000	27	100
1:100,000	24	89
1:500,000	12	44
1:1,000,000	3	11
1:5,000,000	0	0

of Ringer's solution kept at 38° C. by thermostatic control and oxygenated by a constant stream of air. This stream of air also made possible a rapid and uniform mixing of any solutions added to the bath. After the test tissue had remained in the bath for a period of five to ten minuutes, 1 c.c. of the tenth washing of the mold in question was added. Two to three minutes later, 1 c.c. of the fourth washing was added. This was followed after comparable intervals by 1 c.c. of broth (or first washing) and finally by a similar amount of 1 per cent peptone. The method employed in testing the mold extracts was essentially the same as that described for the washings and broth; however, the volume of extract added to the bath was 0.5 c.c., rather than 1.0 c.c.

The solutions were first tested separately on fresh muscle segments and finally, for sake of comparison, with other solutions of a similar nature on the same target tissue. On a separate test basis, each solution was examined at least four times, some more often, as slight spontaneous changes in tone or activity, which always occur even under ideally controlled conditions, were occasionally found to develop after addition of the solution. To rule out the possibility of positive responses, the solutions were rerun several additional times until unequivocal results were obtained.

Having examined the solutions separately, each of the five extracts for any one mold preparation was tested with similar extracts on the same muscle segment. It was thought that slight differences in histamine content might be detected by this procedure which would not be evident otherwise. By varying the sequence of addition, each extract was com-

pared with other extracts, similar in source but differing in method of extraction, on a common target tissue.

The sensitiveness of the target tissue was finally demonstrated in each instance by characteristic smooth muscle contractions produced by the addition of 0.05 c.c. or 0.1 c.c. of a 1:100,000 dilution of histamine to the bath. In most instances, the tissue responded to the smaller amount of histamine, which represented a bath dilution of 1:300,000,000. In case of doubtful responses to the solutions tested or poor responses to the standard histamine solution, the test was repeated on fresh tissues.

Testing for Sensitiveness of Skin to Histamine.—In order to determine whether or not the normal human skin might respond to amounts of histamine too minute to give positive reactions by the guinea-pig method, normal subjects, ranging in age from five to fifty years, were tested intracutaneously with 0.02 c.c. of the following histamine (base) solutions: 1:50,000, 1:100,000, 1:500,000, 1:1,000,000, 1:5,000,000. Physiological saline, with which the histamine solutions were prepared, was used as a control. Reactions were read and recorded according to Cooke's classification for intracutaneous testing.

RESULTS

Schultz-Dale Technique.—While the addition of a given solution was occasionally followed by a change in tone or temporary decrease in activity, such a change was shown to be spontaneous and not due to constituents of the solution. Questionable responses were not duplicated on re-examination.

The results indicate that the tenth, fourth, and first washings (or broth) of the various molds were uniformly negative as regards their ability to cause characteristic histamine-like contractions of the uterus or intestine. Likewise, the addition of concentrated (1:20) mold extracts, prepared by the five different procedures, failed uniformly to produce specific reactions.

Skin Tests with Histamine.—The response of normal individuals to intracutaneous injections of histamine varied markedly. While none of the twenty-seven subjects reacted to a dilution of 1:5,000,000, three (11 per cent) responded to 1:1,000,000; twelve (44 per cent) responded to 1:500,000, twenty-four (89 per cent) to 1:100,000, and all twenty-seven to 1:50,000.

DISCUSSION

Failure to find histamine or a histamine-like substance in the extracts does not, of course, rule out the possibility of the presence of other irritating substances. Data on the chemical analysis of dried molds are meager. Those available fail to reveal known irritative constituents which, under the conditions of extractions employed, could be removed in sufficient concentration as to produce reactions in normally sensitive individuals.

Protein, according to Cramer[4], is present in molds in relatively high concentrations (30 to 45 per cent), but its nature is not definitely established. It is known, however, that most fungi contain a variety of different enzymes[10], some of which are proteolytic; these make possible the presence of peptone and other products of protein hydrolysis which may be irritants. Most of these enzymes, presumably, are inactivated by drying and extraction, but there is no reliable information that this is actually so. One enzyme, histaminase, has been found in a number of molds, including the aspergillus group[20]; this enzyme may account for the absence of histamine in the extracts.

Peptone, either in the form of a residuum of the culture-broth or in the form of mold protein, does not itself seem to be the likely irritant in the concentrations in which it exists in the extracts. Although commercial peptone has been reported to produce wheal formation in the human skin in a 1:100 concentration, and contraction of the guinea-pig uterus in a concentration of 1:1,000, such results, according to some workers, are due to contamination with histamine. Hanke and Koessler[11] found as much as 0.003 gm. of histamine dichloride per 100 grams of peptone in some commercial preparations. Using Bacto peptone the writer has failed repeatedly to obtain positive skin reactions with a 1:100 solution and contractions of the guinea-pig intestine with a bath concentration of 1:500.

Besides the better known hydrolytic products of proteins which may be present in the extracts, there may also be toxins of highly complex and unknown chemical composition. Various endotoxins, of course, are found in certain fresh fungi; these are effective in minute amounts. Nothing, apparently, is known about their presence and activity in dried forms subjected to extraction with a higher alcohol. There seems to be a need for fundamental studies of these endotoxins along the lines accomplished by Waksman and his associates[19] for actinomycin A and by Robinson and Moliter[16] for tyrothricin.

In attempting to account for the so-called "irritative" and nonspecific reactions of the extracts in the absence of known toxic substances, it might be emphasized that the skin of normal individuals varies greatly in response to a variety of "stimulants." As a result of trauma by physical or chemical agents there is supposed to be liberated either histamine or a histamine-like substance (H-substance of Lewis) which acts upon the cutaneous vessels to produce the wheal and surrounding flare. Such a reaction can be produced by the injection of a number of chemical agents (such as codeine, morphine, atropine and various ions) and by mechanical means. Injury, whether produced physically or by nonspecific chemical irritants, results in reactions which cannot be differentiated from specific reactions produced by allergens.

In returning to the failure to find histamine-like substances in the mold extracts, it is emphasized that the smooth muscle segments used as

target tissues responded to histamine concentration of 1:150,000,000; many reacted to 1:300,000,000 and a few to 1:600,000,000. Such concentrations are approximately one thousand times less than the average minimum concentration necessary for wheal formation in the normal human skin. If the mold solutions in question give nonspecific reactions due to the presence of undetectable histamine, individuals so reacting must therefore be 1,000 times more sensitive than the guinea-pig ileum—the most sensitive tissue to histamine yet known. This does not seem likely.

SUMMARY AND CONCLUSION

It has not been possible to demonstrate the presence of histamine or a histamine-like substance in the washings of *Aspergillus niger* or *Alternaria tenuis*, or in the broth on which these molds have been cultured. Nor has it been possible to detect histamine-like substances in concentrated extracts of these molds employed in skin testing.

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MOLD FUNGI IN THE ETIOLOGY OF RESPIRATORY ALLERGIC DISEASES

III. Immunological Studies with Mold Extracts

4. Skin Tests with Broth and Washings from Mold Pellicles

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FOLLOWING the failure of Selle to demonstrate by animal experimentation the existence of histamine-like substances in the broth in which *Alternaria tenuis* and *Aspergillus niger* pellicles had been grown, in the saline washings of these pellicles, or in the allergenic extracts prepared from them, it seemed appropriate to study further the broth and washings by testing them on the human skin. However, inasmuch as no patients were available in whom sensitization to *Aspergillus niger* was considered significant this study was limited to the broth and washings of *Alternaria tenuis*. Nine individuals with a negative history of allergy were tested by the intradermal method with routine Alternaria extract 1/1000 and 1/100 and with the twentieth, fifteenth, tenth and fourth saline washings from the pellicles and with the culture broth itself. Eight of these normal controls were likewise tested with varying dilutions of histamine base.

Thirteen patients with respiratory allergy in whom Alternaria was considered an important causative factor were likewise tested with the washings and broth. In three of these allergic individuals the testing could not be carried out all the way down to the broth, in one case because the testing produced a systemic reaction and had to be discontinued.

Table I shows the result of testing in the nonallergic series. No significant reactions were obtained with the Alternaria extracts or with any of the washings or broth. The eight individuals of the control series tested with histamine gave positive reactions.

Table II shows the results of testing in Alternaria-sensitive patients. It is significant that in every instance in which it was tested the broth gave strong reactions. Furthermore, the washings likewise gave definitely positive reactions in nine of the patients, and precipitated asthma in one instance (Case 6).

DISCUSSION

In this small series of nine normal individuals, Alternaria extracts as well as saline washings from the pellicles and the broth in which the pellicles are grown do not appear to be skin irritants. On the other hand, washing the pellicles in saline solution prior to extraction seems to remove at least a portion of the substances capable of producing positive skin tests on Alternaria-sensitive patients. This observation is offered as a possible explanation of the lack of potency in previously prepared extracts.

Presented before the Association of Allergists for Mycological Investigations, St. Louis, Missouri, January 21, 1944.

TABLE I. RESULTS OF SKIN TESTS IN NINE NONALLERGIC INDIVIDUALS

Case	Alternaria 1/1000	Alternaria 1/100	Washings Alternaria SB-6					HISTAMINE BASE			
			20	15	10	4	Broth	1/1,000,000	1/100,000	1/10,000	1/1000
1 Mrs. T.P.P.	–	–	–	–	–	–	–	–	?	++	+++
2 R.H.	–	–	–	–	–	–	+	–	–	–	+++
3 F.A.H.	–	–	–	–	–	?	+	–	?	++	+++
4 L.T.	–	–	–	–	–	–	–	–	+	++	++
5 E. H.	–	–	–	–	–	–	–	–	+	–	+++
6 W.S.	–	+	–	–	–	–	–	–	+	–	+++
7 Mrs. B.W.	–	–	–	–	–	–	–	–	–	+++	
8 Mrs. M.D.P.	–	–	–	–	–	–	–	–			
9 Mrs. R. E. D.	–	?	–	–	–	–	–	–	?	++	

TABLE II. RESULTS OF SKIN TESTS IN THIRTEEN ALTERNARIA-SENSITIVE PATIENTS

Case	Alternaria 1/1000	Alternaria 1/100	Washings Alternaria SB-6				
			20	15	10	4	Broth
1 H.D.		++	–	?	?	+	+++
2 J.S.	+++	++++	+	+	+	+	+++
3 S.D.	++	++	+	++	++	++	++++
4 S.R.R.	++++	++++	++++	+++			
5 M.S.W.	++	++++	+	+	+	+++	++++
6 S.W.	+++	+++	++	+++	++		
7 E.S.	++	++++	++	+++			
8 J.L.	+	+	++	++	++		
9 S.C.		+++	+	+	+	++	+++
10 C.M.		++++	++	++	+++	++++	++++
11 Mrs. D.S.	+++		+++	+++	+	+++	++++
12 P.W.	++	+++	?	?	+	+	++++
13 D.G.	++	+++	+	+	+	+	++++

MOLD FUNGI IN THE ETIOLOGY OF RESPIRATORY ALLERGIC DISEASES

IV. Skin Reactions to Molds as Correlated with Relative Importance in Treatment

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THE data presented herewith have been gathered during three years of testing and treating allergic patients to the air-borne molds. The group includes 705 patients from San Antonio and an approximate radius of fifty to seventy-five miles. All of the patients were tested with a 1-100 dilution of the concentrated mold extracts as prepared by the Association of Allergists for Mycological Investigations, using the intradermal method. Alternaria, Hormodendrum, Penicillium, Helminthosporium and Aspergillus were used.

The 705 patients are classified according to allergic manifestations as follows:

234 (34 per cent) with asthma primarily.
396 (56 per cent) with hay fever.
18 (2.5 per cent) with sinusitis in which an allergic component was suspected.
10 (1.6 per cent) with headache in which an inhalant component was suspected.
5 (0.7 per cent) with allergy of the eye.
30 (3.8 per cent) with allergy of the skin or cases in which the diagnosis of allergy was not absolutely clear.

The group included all ages from three months to eighty-three years. There were 404 females and 301 males. The numbers and percentages of patients who reacted to and were treated with the various molds may be classified as shown in Tables I and II.

Two hundred and ninety-eight (41.7 per cent) did not react to any of the molds. 163, or 23 per cent, reacted to one mold only. One hundred and one (14.5 per cent) reacted to two molds. Eighty-eight (12.5 per cent) reacted to three molds; forty-six (6.5 per cent) reacted to four of the molds and only eighteen (2.5 per cent) reacted to all five molds.

In correlating treatment with the molds it was found that of the 144 patients treated with Alternaria, sixty-eight (40 per cent) were treated only with Alternaria, while 60 per cent were treated with Alternaria in combination with one or more of the other molds. Of the forty-six patients treated with Hormodendrum, nine (20 per cent) were treated only with Hormodendrum, with 80 per cent treated in combination with one or more molds in addition to Hormodendrum. Of the twenty-six patients treated with Penicillium, only four (16 per cent) were treated with Penicillium alone. Of the eighty patients treated with Helminthosporium, twenty (25 per cent) were treated with Helminthosporium alone, leaving sixty (75 per cent) treated in combination with the other molds. Of the

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TABLE I. SKIN REACTIONS TO MOLDS

Molds	Negative		Slight		Moderate		Large	
	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent
Alternaria	370	52.9	130	17.7	113	16.4	92	13
Hormodendrum	489	69.3	120	17.0	84	11.8	12	1.9
Penicillium	563	79.0	78	11.4	49	7.5	15	2.1
Helminthosporium	454	64.4	98	14.0	87	12.3	66	9.3
Aspergillus	462	66.7	130	17.7	90	12.7	23	2.9

TABLE II. PATIENTS TREATED

Molds	Number	Per Cent
Alternaria	144	20
Hormodendrum	46	6.5
Penicillium	26	3.6
Helminthosporium	80	11.0
Aspergillus	44	6.3

forty-four patients with Aspergillus, only five (12 per cent) were treated to that alone, while 88 per cent were treated with Aspergillus with the other molds.

On the basis of reactions to skin testing to the molds and other pollen, food and inhalant allergenic factors, and the subsequent clinical course of the patient, it was felt that the molds were probably of no importance clinically in 524 (74.5 per cent) of the patients. It was thought that they were of slight importance in sixty-seven (9.5 per cent) and of appreciable clinical importance in 114 (16 per cent) of the patients. Therefore, it was concluded that the air-borne molds were clinically important in 25.5 per cent of the patients tested and treated.

It might be important to mention that mold counts on vaseline slides exposed over a period of two and a half years have shown that there is no time during the year in San Antonio in which Alternaria, Hormodendrum and Helminthosporium are not present in the air in some quantity. Alternaria counts average about thirty spores per day with somewhat higher peaks observed in July and December.

Constitutional reactions to Alternaria in the 1-100 and 1-1000 dilutions, Hormodendrum 1-100 and 1-1000, and Helminthosporium in the 1-100, 1-1,000 and 1-10,000 dilutions have been observed.

CONCLUSIONS

1. Air-borne molds were thought to be important clinically in 25.5 per cent of the 705 patients tested.

2. Alternaria was considered the most important of the molds in treatment, with Helminthosporium and Hormodendrum less so.

3. Aspergillus and Penicillium were found to have little clinical significance in the treatment of allergic diseases.

FOOD ALLERGY

II. The Technique and Clinical Application of Individual Food Tests

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THIS communication is concerned with the presentation of the clinical use of individual food tests, in conjunction with skin tests, in the diagnosis of food allergy. It is based upon information gleaned in a large series of tests performed by the technique reported herewith, over a period of ten years with essentially the same method. Much of the information was obtained by making twenty-four hour observations on an original patient for six consecutive months and two subsequent patients, one of whom was kept under such study for six weeks, while the third was observed in like manner for four months. I should like to emphasize that more was learned, concerning the use and value of food tests, from these three controlled patients than from all the other studies.

There is need for a correlated exposition of diagnostic measures other than skin tests since none of the textbooks on allergy, at this time, present such detailed procedures co-ordinated for clinical application.

I. PREREQUISITES FOR THE USE OF INDIVIDUAL FOOD TESTS

The first, and an absolute, prerequisite for the application of the procedures outlined herewith, other than skin testing, is that the patient, if his symptoms are not wholly due to foods, is fully protected against all inhalant factors. While this statement implies a considerable amount of detailed work which cannot be discussed in this paper, it should be mentioned that this infers the patient's degree of sensitivity will have been determined and that the treatment dose has been governed by the degree of sensitization for each of the specific inhalants. The necessity for protection against inhalants is not based upon any presupposition that all symptoms not controlled by inhalants are necessarily due to foods, but to prevent error, in the individual food tests, in those patients sensitive to inhalants (possible contact during test).

The second requirement for the use of these diagnostic measures is that the physician be thoroughly acquainted, both didactically and clinically, with the nature and dynamic mechanism of food allergy.[9] This means that the physician should perform these tests himself until he is entirely acquainted with all these factors.

A third and important fact to keep in mind is that, with present technique of testing, there is no single method, or combination of methods, of testing a food on one occasion, which will yield information that becomes a permanent guide as to the exact subsequent use of that food by the patient.

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II. THE TECHNIQUE OF INDIVIDUAL FOOD TESTS

The method of testing foods individually, as described in this article, was originated in October, 1932, and by October, 1934, the technique was standardized and has remained practically constant since that time. While more than 25,000 tests have been made by this method, only the exceptions to the rule were discovered after the first 500 were performed.

Preparations Preliminary to the Test Day—The food to be tested should be used regularly in the diet for at least two weeks preceding preparations for the test. It should then be avoided exactly four days and tested on the fifth day.

Preparations of the Food Used for Tests.—The food must be prepared individually without added condiments, sugars, et cetera, using an average portion which is to be eaten within five minutes' time. Should the food fail to produce definite symptoms, at the end of one hour repeat the feeding, using one-half portion.

Instructions for Patient on the Test Day.—The patients are instructed to take neither food nor drink for five hours preceding the feeding time. They are to avoid all medicine for four hours and drinking water and smoking for three hours immediately preceding the start of the test. Be sure to ascertain, before starting the test, if these instructions have been carried out.

The Objective and Subjective Studies of the Tests.—The following are the objective and subjective studies which are made as part of the individual food test. These observations are made for one-half hour before the test, for one hour after the first feeding, and for one-half hour after the second eating of the food. The patient must be kept in a constant environmental status, i.e., avoiding exercise, argument, drafts and change in posture.

1. Observe the patient for:

(a) Nasal symptoms: sneezing, watering, itching and blockage

(b) Chest symptoms: clearing of the throat, coughing, wheezing

(c) Inquire concerning the occurrence of tiredness, headache, pain of any description, pruritus of the skin, lips, palate, throat, et cetera, hives, gastro-intestinal symptoms, such as nausea, bloating, cramps, retasting, et cetera. Pay particular attention to the occurrence of a "chilling sensation." It may be overlooked if one is not aware of its occurrence. Observe for perspiration, without other adequate cause.

(d) Make delayed observations of a comparative nature, i.e.: compare symptoms during the night after the test and the following morning, with the condition existing the night and morning just before the test. In order to make observation "d" of any value, it is imperative that a patient not use any food the day of the test except the test food, unless it was eaten the day previous.

2. Record the following:

(a) The pulse and respiration rate, as well as the blood pressure, at the end of the thirty-minute rest period before eating. Also take these readings at twenty, forty and sixty minutes after ingestion of the food, with a final reading at the end of the test, that is, thirty minutes after the second feeding.

(b) Take leukocyte counts at the end of the thirty-minute rest period, then again at twenty, forty and sixty minutes after the initial ingestion of the food.

(c) Make total eosinophile counts at the same time that the white blood counts are taken.

(d) In the thermal types of food allergy, subject the patient to chilling following the test. All these procedures are to be carried out in the office, under the direct observation of the physician.

Originally, when I began the individual food tests in October, 1932, only the preparations for the test and the first three steps were carried out.

In 1933 (when Vaughan[11] described the leukopenic index, blood counts were added to the studies. It was found that the criteria of blood count changes for sensitization as advocated by Vaughan's first paper, did not entirely coincide with the clinical findings and the counts were discontinued temporarily. In October, 1934, the leukocyte counts were added again to the studies when it was observed that a trajectory type of curve, with postprandial counts, made at twenty, forty and sixty-minute intervals, coincided with compatible foods. These observations were made in a patient with extreme asthma who was observed twenty-four hours daily, for six weeks. There was no possibility for error in ingestion nor did any symptoms occur which were unknown to me. The patient was not only freed of symptoms when the diet was limited to the foods giving this type of curve, but has continued to remain free of symptoms for ten years except for deliberate dietary tests or accidental ingestion of foods. It seems reasonably safe to assume that this curve indicates a food that is nonallergic. Furthermore, this possibility has been studied in many subsequent instances, and it yet remains to be proven that a food, properly tested, which gives a trajectory type of leukocyte curve, *is not compatible under conditions of the test.* The hypothesis that definite leukopenia is evidence of food disagreement has been found to be much more valuable than the contention that if a food did not produce a drop of at least 1,000 cells, it was not a cause of symptoms. Gay[5], in a paper on peptic ulcer, indicated that foods which produced a drop of only 300 cells would sometimes be a cause of allergy. Editorially, the American Medical Association[3] criticized this, stating that blood cells could not be counted within an accuracy of 300 cells in successive counts, missing entirely the obvious implication of Gay's statement. Gay was well acquainted with the limitations of accuracy of counting blood: he was calling attention to the fact that foods which did not alter the count by as much as 1,000 cells were ac-

tually the cause of symptoms, and one could not assume that when the leukocyte count did not drop 1,000 cells, that the food was compatible.

At the present time the value of the leukocyte counts lies in the finding of (1) compatible curves[10] and (2) in the finding of marked leukopenia without the association of symptoms. In these latter cases, one is to be on the alert for two facts: (1) the occurrence of delayed reactions, ten to fourteen hours after eating and (2) the probability of cumulative reactions (Phase III).[9] This will be discussed, under clinical application of tests. Wide fluctuations of the counts are highly suggestive of food disagreement.

The final additions to the food test were the inclusion of the pulse rate[2], as a routine measure in 1942, and the total eosinophile count, using Randolph's[7] technique in the past year

The study of thermal factors was started in the fall of 1937.[4] This is best done by exposure to air below the critical temperature for a patient or by approximating chilling by means of ice, or immersion of the hands in ice-cold water. Thus, except for the total eosinophile counts, and the routine study of the pulse rate, there has been no change in the technique of the test during the past seven years.

There are a number of points which should be stressed concerning the clinical use of these tests:

First, if a patient cannot be freed of symptoms after making individual tests of the ten to twelve most common articles in the diet, there is no real cause to believe that the performing of sixty to one hundred tests will relieve the patient.

Second, clinical advice to the patient must be made in terms of the previous use of the food and the type of food sensitization involved. For example, if a food is found compatible on testing, it must be subsequently determined whether this is a perennial, a co-seasonal or a thermal type of food allergy. If the food has been eaten three times weekly before testing, the patient is to be advised to continue using it with this frequency. The patient may develop an allergy to this food if the incidence of ingestion is increased (Phase III).[9] If the test is made in July, the patient cannot be assured as to the effect of the food during the ragweed season or during the winter months as concomitant inhalant factors occur in pollen seasons and thermal effects in winter.

Third, food tests, properly performed and interpreted, will be diagnostically accurate in approximately 90 per cent of the tests. It should be emphasized that the selection of cases, i.e., perennial nasal allergy, asthma or migraine, et cetera, will determine, to a great extent, this average accuracy. The figures given here are made up chiefly from patients with perennial nasal allergy, asthma and seasonal hay fever, with a small per cent of migraine, eczema, hives and gastro-intestinal allergy.

Fourth, patients improve with the elimination of foods whose sensitization can be proved by this type of test. The greatest value of individual

food testing is the clinical observation of the effects produced by a food in the test period. This study has been neglected apparently by most students of the individual food test methods. It is absurd to have a patient in the office for two and a half hours for blood counts, and to attempt to make a diagnosis by use of these alone, when this same period of time may be used for the purpose of clinical observations if the patient has been properly prepared. During the first two years, the test procedure consisted of clinical observations alone, blood counts being added in 1934.

INTERPRETATION OF CLINICAL FINDINGS

Symptoms of Sneezing, Watering of the Nose and Coughing.—These are recorded as they occur for one-half hour before eating and for the duration of the test. Patients are advised not to blow their noses until the secretions are profuse enough that the nose will drip. This is considered a unit, i. e.: one watering of the nose. Patients should avoid "sniffling," but if they do so, or in the young, this may be taken as a unit, but must be used as such throughout the whole test. Sneezing is recorded as it occurs. Both coughing and clearing are registered under coughing. Itching of the nose, or any other type of oral pruritus is elicited at the start of the test, its presence or absence noted and the patient instructed to report its occurrence, as soon as symptoms occur. The patient is questioned for these at the time of each leukocyte count. Nasal blockage is likewise registered and studied throughout the test.

The value of these studies will be in terms of the acuity of the observer, correctness of preparation for the test and, in some instances, the co-operation of the patient. It has been found that some patients knowing that excessive nasal symptoms indicates disagreement of a food, will attempt to subdue their reactions. Children reacting to a food they like often attempt to hold down the normal tendency to cough. The findings are considered significant if the food produces a doubling of the incidence of symptoms. Many times the food causes allergy and will not double incidence; however, this is a conservative rule.

If one uses the incidence of symptoms only as a means of diagnosis, more foods will be eliminated at the start of the study than need to be kept out to relieve the patient. When eliminations are made upon this basis, a recheck should be made as soon as definite clinical improvement occurs, or upon freeing the patient of all symptoms. The clinical value of this test increases as the patient improves. If a person is entirely free of all symptoms before eating, the definite occurrence of sustained symptoms is highly significant.

Among patients who develop post-ingestive symptoms during the first hour, the highest per cent commence to have symptoms ten minutes after eating, the next highest per cent at five minutes, and the remainder will occur throughout the hour, being progressively less, with the increase of time after eating. These studies are among the most valuable of all food allergy diagnostic methods available. They have proved valuable when all

other means have not been of help. They will only be ineffective if one disregards the factors that influence their occurrence.

Symptoms of Tiredness, Pain, Pruritus of Skin, Headaches, Gastro-Intestinal Symptoms of all Types.—These are determined before, during and at the completion of the test. Tiredness is probably one of the most significant symptoms, usually developing within forty-five minutes and often fleeting.

Blood Pressure, Respiration and Pulse Readings.—Blood pressure readings have been made only in patients subject to increased pressure. Some of these cases have been studied for seven years in whom there was a constant demonstrable relation between specific articles in the diet and post-ingestive hypertension.

Respiration will usually increase in rate at the beginning of the bronchial reaction. Since increases in the respiratory rate are seldom seen without other more significant symptoms, this observation is only confirmatory. Pulse readings have been made from time to time, but have been routine for the past two years, chiefly in an attempt to evaluate their use. At this time it may be stated there are (1) patients who have no other test period findings of reaction than an increase of the pulse rate, and (2) there are many food allergies not associated with changes in the pulse rate, (3) that post-ingestive tachycardia is not the rule with agreeable foods and (4) that the most valuable feature of the pulse increase is to suggest the occurrence of delayed reactions ten to fourteen hours after the test. Thus, this feature is, along with marked leukopenia without any symptoms, the signs for alertness in observing patients as to delayed effects from the food being tested. This should always be done, of course, when neither of these findings is present.

*Leukocyte Counts.**—These are made with the same pipette for the same patient, the pipettes are shaken in a mechanical shaker and eight large squares are counted on the same chamber each time. Persons making counts should check their technique by using two pipettes and graphing their results. These must be consistent within the allowed error (5 per cent), in counting and a cross test with another technician should also be made to eliminate the possible identical error one would make with both counts. There has been no change in leukocyte counts since that described in my most recent article on the subject.[8]

The use of leukocyte counts has been subject to criticism[1,6]. That one could seriously and consistently use this test, ignoring the clinical possibilities that obtain at the time of the test, seems unbelievable, but this is the only possible conjecture from these reports. There are only two directly usable features of the leukocyte counts alone, namely: (1) the finding of the trajectory curves, which may be taken to indicate a compatible

*Clinical findings are recorded on a special chart which is included in the author's reprints.

food, for purposes of clinical application, and (2) the finding of definite leukopenia without any other evidence of disagreement. In this latter case the suggestion is that the patient is apt to have delayed symptoms. Total eosinophile counts, using Randolph's[7] method, have not been used sufficiently long to give us definite interpretive information.

Thermal Tests.—It is obvious that since patients with this type of allergy[4], will have only their major symptoms during cold weather, it will be quite easy, as a rule, to subject the patient to normal exposure to cold air. If this is not possible, then simulate with ice or ice-cold water the chilling effect and observe the patient thereafter for one-half hour or an hour, depending upon the circumstances.

The occurrence of "chilling sensation" following the eating of foods occurs in many patients who are not major cases of thermal allergy. This symptom may be elicited the same as tiredness.

CRITERIA FOR THE SELECTION OF FOODS TO BE TESTED

If a patient, after he has been adequately tested and treated for inhalant allergies (this would not be true in migraines and gastro-intestinal patients as a rule), presents a chain of symptoms which are either typical of food allergy or could in all likelihood be due to foods, then certain articles in this patient's diet are selected for tests. This selection is based upon probability.

Probability has been determined by the analysis of several hundred patients in whom symptoms did not recur except with deliberate ingestion. It was found that wheat, egg, milk, corn, oranges, tomatoes, string beans, Irish potatoes, et cetera, was the numerical order of frequency of foods causing symptoms. Therefore, a patient who failed to react upon skin testing, or who failed to be relieved of his symptoms when placed upon a diet based upon positive skin tests for foods, in conjunction with specific and correct inhalant therapy, wheat should be tested first. On the basis of probability, it is the most likely food to be a cause of symptoms. In the selection of foods for individual testing, one must bear in mind that a patient usually consults him for a masked food sensitization. For example, if a patient knows that every time he eats onions he is sick three to five days, he will stop using onions. He comes to you for help because he has symptoms like these created from eating onions but whose occurrence does not seem to bear any relation to a given food, or else he thinks he is sensitive to all foods. This is a typical story of a patient with masked food allergy. For this reason, no matter how thorough a history may be, it cannot be used to diagnose the specific food allergy as some advocate.

The selection of foods for testing will be discussed for each disease in particular, in order to detail minor syndrome characteristics.

1. *Asthma.*—The following are the clinical characteristics of existing food allergy in a patient properly treated for inhalant sensitizations:

(a) The production of large quantities of mucus. While inhalants may produce copious quantities of mucus this will not be true for the inhalant treated individual unless he contacts a rare inhalant and then the history of the attack should be diagnostic (flour mill dust, et cetera).

(b) The occurrence of acute attacks lasting from two to five days, the latter only in association with purulent secretions, when there have been no essential changes in environmental contacts.

(c) In patients who live in the same house, sleep in the same bed and work at the same job, acute attacks commencing in the middle of one night of the week are so typical of food that they are considered, for the purpose of clinical procedure, as being due to foods until disproved.

(d) Either of the attacks as described under "b" and "c" may be superimposed upon another characteristic pattern of food allergy, namely, the patient who has difficulty, in spite of treatment, from the time he first awakens until an hour or two after breakfast, or even as late as 11:00 a.m. and then feels quite well the remainder of the day.

(e) The most typical, but not necessarily the most common, is the occurrence of asthma every afternoon about 4:00 to 5:30, not related to, or affected by, other inhalant or environmental factors. This is typical of a food eaten at breakfast and repeated at luncheon.

2. *Perennial Nasal Allergy.*—The five clinical syndromes described with asthma, occur in nasal allergy, except that the symptoms are nasal instead of bronchial and are in the treated individual indicative of foods until disproved. In adition to these, the continuation of nasal occlusion in a treated patient, must be taken as a sign of food complications again, until disproved. One will often see patients with this syndrome in whom there are either no, or very few, inhalant sensitizations, and in whom all skin tests for foods are negative. These patients are primarily problems of food sensitization. In connection with this statement I would like to emphasize, however, that in sixteen years of allergic practice I have found only two patients in whom all symptoms of respiratory allergy could be controlled by diet alone. In all the others, there was a mixture of sensitizations of foods and inhalants.

3. *Migraine.*—The selection of foods for testing in patients with migraine, is determined by the type of clinical symptom pattern. In the first the patient would have intermittent paroxysms of typical symptoms, two to three days in duration, spaced at various intervals. Between these attacks he is entirely free of symptoms, which implies absolutely no pruritus of the nose, roof of mouth, nor any tiredness nor tachycardia. The second type of patient is the one who is never free of headache, pruritus and tiredness. This person has superimposed on his constant level of symptoms acute exacerbations lasting two to three days. In the first instance, the diagnosis can certainly be suggested by the diet record alone, but it must be substantiated by the individual test. The diagnosis of the specific

effect of a food is never made by improvement following its elimination. It must always be made upon the basis of the effect produced by the proper reintroduction of the food to the diet (food test). In the second group of patients we are dealing with foods used constantly as well as those used intermittently. In the study of migraine one must consider tiredness as the predecessor of headache. A food which is taken every three or four days and produces only tiredness will, if used repeatedly, produce headache.

If the skin tests have not been successful in detecting the cause of a constant pattern of symptoms, then individual tests are to be made, based again on probability. Therefore, it is imperative that early in the diagnosis of these cases that adequate diet records are kept although most patients will not deviate greatly from the common probable causes, but there may be exceptions, as, for instance, a patient extremely fond of asparagus, or a patient who always insists on eating avocados, or who constantly uses prunes. Exacerbations in migraine patients can be due to two things: either the increased use of one food or the occasional use of a specific food. For example: a patient may eat peanuts once every third day and will be subject to more or less constant tiredness, but if they are used three or four meals in succession, will have a very definite headache. These two conditions will only be revealed by a diet record. Headaches due to foods may occur during catamenia only.

4. *Eczema and Hives.*—The selection of foods for testing in these patients is not greatly different than the other allergic diseases except that in urticaria the duration of food symptoms may be much longer than in either asthma or hay fever. In the case of eczema, pruritus (not pathologic changes in the skin) is to be used in the determination of offending foods. Thus, a patient with food eczema continues to suffer from pruritus only as long as he continues to ingest products to which he is sensitive. Pruritus usually subsides in fifty to seventy-two hours after the last eating of the food to which the patient is sensitive, but the eruption may not clear up for as much as twenty days afterward. In the patient with eczema, pruritus when due to a food used constantly will show exacerbations late in the morning but, more typically, late every afternoon as well as in the middle of every night. This is all evidence of a food used constantly in the diet.

5. *Gastro-Intestinal Allergy.*—The selection in these patients needs little emphasis except for two points.

(a) In a patient who has a probable gastro-intestinal allergy, one should bear in mind that sensitization is only one of many probable causes of such gastro-intestinal symptoms and, therefore, a complete differential diagnostic study is imperative.

(b) With patients treated for ulcer who have not responded to the treatment, the food used constantly in the treatment of ulcer assumes more

importance as a likely cause of trouble and probability would differ, therefore, from the general rule. In other words, milk, instead of being the third most likely food to cause symptoms, would be the most likely; then egg, cereals, et cetera.

CLINICAL APPLICATION OF THE TEST

The test should be performed with due regard to the nature and the dynamic mechanism of food sensitization. This means that one must create by immediate avoidance of the food, the condition for maximum clinical response, for it is the clinical evidence (symptoms) that constitutes the greatest value of the test. Further, the food must not be out of the diet long enough for the patient to gain partial tolerance (Phase II).[9] Patients who gain tolerance, that is, pass through Phase II of the cyclic changes within a short time, may give an asymptomatic response to the food if the test is made after elimination of the food for longer periods of time. Whenever a patient is tested and is found not sensitive to a food, the use of the food in the future must be in terms of the immediate past. For example, if one tests a food that is used but once in ten days, and it is found to be compatible, you can advise its use only once every ten days in the future, with assurance that under the conditions of the test (coseasonal or thermal), the food will remain compatible. Therefore, it is not very wise to check foods individually unless they normally are used almost daily. It is necessary to recheck a food, if one wishes to change its use from once bi-weekly to daily. One cannot assume by means of any test method, that it will remain agreeable for the patient, if the frequency of the ingestion is increased. It is also imperative to know that a food, tested and found to be compatible, may be added to the diet and used for two or three weeks and then the patient will have an insidious onset of symptoms which will not be immediately related to the ingestion of this food. Yet this food is the cause of the patient's distress. Thus, one must be on the alert for Phase III[9], of the cyclic changes, when changes in the dietary incidence of a food are advised. This change is most likely to occur with foods previously known to be a cause of allergy, which have been omitted for months and are readmitted to the diet follwing rechecking. It is possible for these foods to recreate an allergic status even when used but once every third day and completely mask their ill effect when used at that interval. If foods are tested in a patient who has a history of thermal factors, then they should be rechecked when it is possible to evaluate the thermal factor. It is possible, but not nearly as desirable, to simulate the thermal exposures. Patients who are subject to seasonal air-borne products cannot be advised by tests, run out of season, as to the effect of these foods during exposure to specific seasonal inhalants.

Since there is no perfect individual food test, it becomes obvious that such testing to be of value to the physician and in turn, to the patient, must be a part of a systematic, concise approach to the clinical problem of food allergy. Therefore, these tests will serve best when there is a correlated

application of these various procedures, together with the treatment of other etiologic agents.

The keystone of the diagnosis of food allergy is to have a patient symptom-free, upon a known diet, for a consecutive period of a week or ten days. To the attainment of this, all tests and diagnostic procedures should be co-ordinated. When this has been accomplished one will be able to complete the studies without using additional individual tests.

A brief outline of such method is the following:

First is the use of skin tests, followed by the avoidance of such foods to which the patient has given reactions. If this elimination and the concomitant therapy of inhalants has failed to relieve the patient in whom food allergy could be a factor, further studies are carried out. The need for these tests will be greatly enhanced if the symptoms, observed after the patient has been under treatment, have the characteristic nature and time of reaction frequently seen with foods. When these two facts are considered of sufficient import to warrant the use of individual testing, such tests are to be performed. If the patient is one who has a high degree of inherent tolerance[9], the restricted diet of foods found agreeable are to be used for a period of a week, as a test diagnostic diet. This will be possible more often than not.

In the patient with a low inherent tolerance[9], it is best not to prescribe a restricted diet of a few foods but to employ a diversified rotating diet, checking possible allergies when diet and symptoms records indicate these possibilities.

It is of extreme importance to differentiate first between failure to obtain relief of allergic symptoms due to our inability to make a correct diagnosis and, secondly, from our failure to obtain relief because the patient does not know where foods are contacted. Finally, one must differentiate diagnostic error from therapeutic failures due to non-co-operation of the patient. The end results of the use of any diagnostic procedures in allergy, are not entirely due to the perfection with which the physician applies the method, but are as much, if not more, dependent upon the thoroughness with which a patient adheres to the findings obtained by such test procedures.

SUMMARY

1. The technique of individual food tests has been outlined.

2. It is suggested that this test be employed to confirm all skin reactions.

3. The test should be used to discover the specific effects of various foods before assuring they are compatible.

4. The test is to be used in keeping with the basic nature of food allergy as regards incidence and type of sensitization as well as concomitant or thermal factors.

(Bibliography on Page 517)

THE INFLUENCE OF HYPNOSIS ON PASSIVE TRANSFER AND SKIN TESTS

MICHAEL ZELLER, M.D., F.A.C.A.
Chicago, Illinois

THE psychogenic factor in allergy is receiving increasing attention in recent years. Among the various aspects of the psychogenic viewpoint the work of Clarkson[1] on hypnosis has been cited in the recent textbooks of Feinberg[2] and Vaughan[4] and in an article by Peters[5]. Clarkson reported the following case:

"On preliminary investigation an asthmatic girl, aged eighteen, gave a severe cutaneous reaction to an intradermal test for egg sensitization. A wheal one inch in diameter surrounded by a raised erythematous zone three and a half inches across developed around the site of inoculation. Next day the test was repeated whilst the patient was deeply hypnotized. The suggestion was given that no reaction would occur. The patient was kept under hypnosis for half an hour and the suggestion frequently repeated. No wheal developed and there was no erythematous blush. Sphygmographic tracings of the pulse were taken before and during the experiment. As soon as hypnosis was induced the tracing altered markedly. The excursions became accentuated and more frequent. There was a sharp rise and fall. The pre-dicrotic notch was registered close to the base line. The blood pressure increased from 110 mm. Hg systolic and 65 mm. diastolic to 130 mm. Hg systolic and 75 mm. diastolic. Next the experiment was repeated in the patient's normal state and the original wheal was obtained."

As Clarkson reported only this one case in which hypnosis and suggestion seemed to influence the skin response, and as there is no reference in the literature corroborating such an effect, it was decided to repeat the experiment under various conditions.

METHOD AND RESULTS

Two patients, one with bronchial asthma and one without any allergy neither of whom reacted to skin tests with ingestants or inhalants, were passively sensitized by the intradermal injection into both forearms of 0.1 c.c. of Wassermann-negative serum from a patient known to be sensitive to ragweed. Twenty-four hours later one of the sensitized areas was injected with .01 c.c. of a 1:500 dilution of ragweed extract. A four-plus wheal with erythema resulted in fifteen minutes. Control areas did not react. On the second day, with the patients under deep hypnosis, a sensitized area was injected with .01 c.c. of a 1:500 ragweed extract and repeated suggestion was made that skin response would not occur. Within fifteen minutes, both patients developed a four-plus wheal with erythema identical with the wheal which appeared the pre-

From the Department of Internal Medicine, University of Illinois.
Read before the Chicago Society for Allergy, October 16, 1944.

vious day without hypnosis. The results of rechecks were the same, as were those of a third injection of ragweed extract given an hour after termination of the hypnosis and those obtained when the entire procedure was carried out again. In one of the patients repeated suggestion under hypnosis that non-sensitized areas would react upon injection of ragweed extract failed to produce positive skin tests.

Two patients, one with ragweed hay fever and one with bronchial asthma, presented four-plus skin tests to ragweed extract applied by the scratch method. Both were placed under deep hypnosis during which ragweed extract was again applied by the scratch method and the suggestion repeatedly made that skin response would not occur Within a few minutes erythema and wheal formation developed as before hypnosis. Application of the extract one hour after hypnosis produced a similar response in the same length of time.

A patient sensitive to cat and dog dander presented wheals one inch in diameter upon application of these danders by the scratch method. Under deep hypnosis repeated suggestion was made that the application of cat and dog dander to the skin scratch would fail to elicit swelling and redness. However, the response developed in ten minutes, just as before hypnosis. On the following day a third application without hypnosis again resulted in the wheal formation described. The same result was obtained a second and a third time.

COMMENT

In view of the increasing prominence of the psychogenic aspects of allergy, particularly asthma, it would seem highly important to substantiate this phase on as tangible a basis as possible. Certainly, any procedure that would demonstrate an alteration, abolition, or retardation of the allergic skin mechanism would constitute a definite step in this direction. Furthermore, it would offer clear-cut evidence that psychogenic factors act not merely as a trigger mechanism but influence the mechanism itself. For these reasons the case report of Clarkson would assume considerable importance in opening new avenues of study of the allergic reaction if the findings could be corroborated. Apparently some authors have accepted Clarkson's conclusions on the basis of one case report. We have been unable to demonstrate any influence of suggestion in the hypnotic state on atopic skin sensitivity or skin sensitivity produced by the passive transfer method.

SUMMARY

1. Five patients were studied to determine whether skin responses can be influenced by hypnotic suggestion.

2. Hypnotic suggestion failed to affect the usual response of passively-sensitized skin areas.

3. Hypnotic suggestion failed to affect the skin of patients sensitive to ragweed or to animal dander.

4. According to these results, hypnotic and post-hypnotic suggestion does not influence skin responses.

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FINDING TUBERCULOSIS. Bull. Lederle Lab., 11:19, (Nov.) 1943.

Tuberculosis is a communicable disease which can be prevented, arrested and cured. Diagnostic tests include X-ray, the Mantoux test and the patch test. The Mantoux test will miss few cases of significant infection when used in strengths of 0.1 mg. O. T. or P. P. D. 1 (first strength). In higher strengths, reactions are probably nonspecific. The patch test, as to dosage and test material, is not as well standardized as the Mantoux test. Both tests are good screening procedures for mass case-work. Three methods given for finding cases of tuberculosis are: through the private physician, through clinical services, and through mass examinations or surveys. The use of skin testing, particularly in the latter group, is important. Because of the simplicity of the application, ease with which positive reaction can be read and the effectiveness of selection, the patch test should be used to discover unrecognized tuberculosis. L. J. H.

Food Allergy

(Continued from Page 514)

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Editorial

MAYO CLINIC FELLOWSHIP

The ANNALS is pleased to announce that the Medical Graduate Committee of the Mayo Foundation has approved the placing of a fellowship of the American College of Allergists at the Mayo Clinic and Mayo Foundation. The selection of the person to receive the fellowship will be made by Dr. Charles F. Code, Professor of Clinical Physiology, and the Medical Graduate Committee of the Mayo Graduate School; research work on problems of allergy to be under the direction of Doctor Code in the Mayo Clinic and Mayo Foundation. The stipend for this fellowship will be $1,500 per annum, with provision for a continuation of the fellowship for a second year, if desirable.

The granting of such a fellowship is necessarily postponed until or towards the end of the war, for the reason that Doctor Code at present is engaged full time in war research, and it would be impossible for him to devote sufficient time to satisfactory researches in allergy. Furthermore, it would be difficult at this time to select a highly suitable man for such a position.

Under similar circumstances, the granting of the fellowship to the Standardization Committee of the College,* to be directed by Dr. George E. Rockwell of Cincinnati, may have to be postponed for lack of a suitable candidate. The work of this committee and that of the Laboratory Committee is necessarily delayed, although thorough plans are now being made which will greatly accelerate the work as soon as a qualified person is available and the activities of all can be redirected into fields of allergy research.

F.W.W.

THE ST. LOUIS GRADUATE INSTRUCTIONAL COURSE

The five-day intensive Graduate Instructional Course, held at St. Louis, November 4 to 8, inclusive, by the American College of Allergists was attended by seventy-two registrants, including twelve officers in the Service, from all parts of the United States. The majority of the registrants were diplomates in their various specialties who desire to apply allergy to their practice and have already applied for Associate Fellowship in the College. There are many whose attention was not attracted to the advertisements and news items concerning this course, which appeared in the various national medical journals. As a result, a number who would have liked to register for the course did not do so. Numerous communications have been received at headquarters to this effect.

The course preceded the meeting of the American Academy of Pediatrics and that of the Southern Medical Association. There were day and

*See Annals of Allergy, page 438, September-October, 1944.

evening classes, interspersed by informal discussions, exhibits and practical demonstrations. All registrants were furnished with a set of comprehensive outlines of the lectures, printed on sheets to fit a standard ring book, as well as a mimeographed Manual entitled "Allergy Laboratory and Diagnostic Procedures." Revisions and additions are being made to this Manual, which will be printed and will be available to anyone desiring it.

The instructors for this course, together with the titles of their lectures, appear on page 522 of this issue.

The College is very grateful to the instructors for their untiring efforts and to all those who helped to make the course a success.

F.W.W.

THE INDEX NUMBER—AN APPRECIATION

The index, in this issue, to the contents of the literature published in the ANNALS OF ALLERGY for the past year, deserves particular attention. A perusal will reveal the remarkable scope of investigative and clinical allergy and immunology achieved comparatively recently and since the second world war began. The apprehension of the staff, that owing to the diversion of many scientists in foreign fields there would result a paucity of excellent material, has been unnecessary. Besides the outstanding original contributions listed, the comprehensive reviews of the literature on the various allergic diseases initiated by the ANNALS have attracted much favorable attention. The ANNALS brings to the attention of its readers the gist of the pertinent literature without the necessity of pursuing a maze of bibliography, although the references have been very complete for each review article. Readers are urged to refer carefully to this index before looking elsewhere for any subject pertaining to allergy and clinical immunology.

A feature commencing with this issue is the section of abstracts in Spanish which replace the summaries of the original articles. This feature has been added in response to a request by our Spanish-speaking members. It appears as a Supplement to each issue and will be supplied to subscribers upon request.

The staff takes this opportunity to express its sincere gratitude to the numerous members and friends of the College for their invaluable contributions the past year. We invite your further confidence by asking you to continue this vital interest, and sincerely extend to all the Season's Greetings.

THE SECRETARY

THE SEVENTH ANNUAL FORUM ON ALLERGY

The Seventh Annual Forum on Allergy will be held in the Hotel William Penn, Pittsburgh, Pennsylvania, on Saturday and Sunday, January 20-21, 1945. This is a meeting to which all reputable physicians are most welcome, and where they are offered an opportunity to bring themselves up to date in this rapidly advancing branch of medicine by two days of intensive postgraduate instruction. For in-

stance, the twelve study groups, any two of which are open to him, are so divided that those dealing with ophthalmology and otolaryngology, pediatrics, internal medicine, dermatology and allergy, run consecutively. In addition, the study groups are arranged on the basis of previous registration. In this way, as soon as the registrations are completed, the registrant is expected to write the group leader and tell him just what questions he wants brought up in the discussion. Attention is also called to the fact that during these two days almost every type of instructional method is employed. Special lectures by outstanding authorities, study groups, pictures, demonstrations, symposia and panel discussions.

On Friday evening preceding the Forum, the American Association of Allergists for Mycological Investigation will hold its annual meeting at which time the results of their co-operative research on the Allergy to Fungi will be reviewed. All reputable physicians and scientists are invited to attend this interesting summarization of a year of brilliant co-operative research.

Although the program is most intensive, informality and an emphasis on the practical marks the conduct of the whole meeting. Good fellowship at luncheon, dinner and smoker reigns throughout the two days. Last year, the tradition was established of dining together throughout the meeting, thus offering an exceptionally fine opportunity to meet and come to know many distinguished authorities in this new and rapidly advancing field of medicine.

This international postgraduate Society was founded in 1938 at Cincinnati, Ohio, to provide a place in which to review the progress of clinical allergy, to provide in peace times a Forum for the younger members, and to offer intensive postgraduate instruction in allergy to physicians working in other fields. The founders were Dr. Tell Nelson, Chicago, Illinois; Dr. Karl D. Figley, Toledo, Ohio, and Dr. Jonathan Forman, Columbus, Ohio. Annual meetings have been held each year since; in Toledo, Ohio, in 1939; in Chicago, Illinois, in 1940; in Indianapolis, Indiana, in 1941; in Detroit, Michigan, in 1942; in Cleveland, Ohio, in 1943, and in St. Louis, Missouri, in 1944.

In 1940 the name was changed to correspond to the international character of its attendance and the Forum's Gold Medal and annual oration were established as a means of recognizing outstanding contributions to clinical allergy. The first recipient was Bela Schick, New York City, who introduced the word "allergy"; the second was W. W. Duke, Kansas City; the third, Arthur F. Coca, New York City; the fourth, Robert A. Cooke, also of New York City. This year the Forum medal goes to Milton J. Rosenau, Chapel Hill, North Carolina.

This year the Marcelle prize has been established through the generosity of the Marcelle Cosmetics, Inc., and will be given to the author of the best papers on Allergy appearing in the American medical literature during the year. The first prize will be for three hundred and fifty dollars and the second prize for one hundred and fifty dollars. The awards will be based on the decision of a jury of distinguished allergists.

For further information, copies of the book and registration, write Jonathan Forman, M.D., Director, 956 Bryden Road, Columbus 5, Ohio.

News Items

Dr. F. W. Wittich has been elected an active member of the American Association of Immunologists.

Please make your transportation and hotel reservations now for the Forum meeting to be held at the Hotel William Penn, Pittsburgh, January 20 and 21.

Dr. J. Warrick Thomas, Richmond, Virginia, has accepted a position on the Editorial Council of the *American Journal of Digestive Diseases* at the invitation of Dr. Beaumont S. Cornell, Editor.

Colonel Sanford W. French (MC Ret.) announces the opening of offices at 218 Encino Avenue, Alamo Heights, San Antonio 2, Texas. His practice is limited to the diagnosis and treatment of allergic diseases.

As formerly, all Fellows of the College who are in the Armed Forces have been furnished by Marcelle Cosmetics, Inc., complimentary, a complete set of the printed abstracts of the courses presented at St. Louis. Numerous expressions of appreciation have been received for this generous gift.

Dr. Henry I. Shahon, Chief of the Medical Service and of the Allergy Department of the Veterans Administration Facility, West Roxbury 32, Massachusetts, has been promoted from Captain to Major. Doctor Shahon's book entitled "Compendio de Alergia Clinica," published by Hachette, Buenos Aires, has reached a large sale, and Major Shahon has completed an English edition.

Recently, while in New York, Dr. Fred W. Wittich was presented with a check for $350.00 for the Research Fund of the College, through a grateful patient. The names of the donors are as follows: Mr. Louis Levine, Mr. Julius Duberstein and Mr. and Mrs. Irving Solomon, all of New York City.

The College is deeply grateful for such additional contributions towards the establishment of two Research Fellowships, as announced in this issue.

A very attractive 10-karat gold key, representing membership in the College, is now being manufactured for the members, including Associate Fellows and Fellows. A cut of the same will be mailed to all the members the first of the year. It is expected that some will be available by January 1 for those who wish to place their orders. The price is $10, including mailing. Send orders to The American College of Allergists, 401 La Salle Medical Building, Minneapolis 2, Minnesota.

The annual meeting of the Southwest Allergy Forum will be held at New Orleans Monday and Tuesday, April 9 and 10, 1945. Owing to war board restrictions in war zones, hotel reservations are available only to servicemen and families over week-

ends. This has necessitated a change in the dates of the Forum meeting from Saturday and Sunday, as originally planned.

President Ralph Bowen urges that transportation and hotel reservations be made early. An excellent program is being arranged, a tentative outline of which will appear in the January-February issue of the ANNALS.

Mrs. Ralph G. Mills, of Decatur, Illinois, writes that her late husband's practice and office equipment are available to a reputable physician and allergist who is looking for a location. Besides excellent facilities to practice medicine, Doctor Mills' allergy equipment is very complete, including much dependable material for testing and treatment. Among his numerous attainments, Doctor Mills was a botanist, and his oil extracts for patch testing are unusually complete. Any allergist returning from Service would step into a big vacancy here and render a great service to his patients when attempting to exemplify our late Fellow.

Complete sets of the instructional courses, presented at the first annual meeting of the College in Chicago, June 10 and 11, 1944, are no longer available. The second set of the printed instructional courses, presented at St. Louis, November 4 to 8, inclusive, are now available to all who desire them. These are much more comprehensive and inclusive. The mimeographed Manual of Allergy Laboratory and Diagnostic Procedures will also be printed and the sheets punched to fit a standard ring book so that revisions or additions may be made. The latter will be available very soon. A descriptive advertisement of the course appears in this issue. You are urged to place your order early.

The Committee on Graduate and Undergraduate Education of the College is arranging a Speakers' Bureau. Printed forms of registration for this Bureau will be sent to the members. In this way, a list of speakers would be made available when the Committee plans the subject matter and leaders for the regional instructional courses and the presentation of papers at regional meetings of the College. It will also be useful when giving information concerning speakers to the program committees of county, state, district and other recognized medical societies. Anyone wishing to participate could list the subject he wishes to present, the investigative work done on the subject, and former and present teaching positions. On this registration blank could be listed films which any members wish to loan to the College for these regional instructional courses.

SPECIAL ANNOUNCEMENT

Beginning with this number, an innovation is being made whereby the Spanish abstracts of all the scientific papers contained in each issue of the ANNALS will be published as a supplement to the ANNALS. By publishing the Spanish abstracts as a supplement, considerable space will be reserved within the ANNALS itself, and thereby a saving of paper will be accomplished, as the supplements containing the Spanish abstracts will be mailed to only those subscribers who desire them. If this policy proves popular with the Spanish readers, it is hoped that it may be continued.

✶ *In Memoriam* ✶

RALPH GARFIELD MILLS

Dr. Ralph Mills of Decatur, Illinois, died October 17, 1944, at the age of sixty-three years. He was born at Lincoln, Illinois. He received his A.B. in 1903 from Illinois, and his M.D., *cum laude*, in 1907, from Northwestern. He helped to build and was head of the Kennedy Hospital in Korea, 1908-1912; professor of pathology and bacteriology and head of the clinical laboratories and research department, Severance Union Medical College, Korea, 1913-1918. Later, in succession, he was in charge of the departments of pathology at Peking Union Medical College, China, and of the University of Colorado and the University of Minnesota Graduate School. He was a member of the American Association of Pathologists and Bacteriologists, Wisconsin Academy of Science, American Medical Association, American College of Allergists, Chicago Society of Allergy, Alpha Omega Alpha, Sigma Xi, and other organizations. He is survived by his wife, the former Mary E. Bumgarner, and a son and daughter.

Doctor Mills was a man of the highest integrity and was a friend of all of us. We shall miss him.

LEON UNGER.

INSTRUCTIONAL COURSES AVAILABLE

Sets of the complete intensive instructional courses covering all phases of important allergic diseases, presented at St. Louis, November 4 to 8, inclusive, are now available. They include comprehensive outlines and lectures including tables, figures, diets, prescriptions, etc., with space for additional notes.

Subjects and authors are listed below:

Dermatologic Allergy—Rudolf L. Baer, M.D., New York, N. Y.
The Physiologic and Immunologic Aspects of Allergy (Illus.)—F. W. Wittich, M.D., Minneapolis, Minn.
The Diagnosis and Treatment of Allergy of the Nose and Paranasal Sinuses—French K. Hansel, M.D., St. Louis, Mo.
Some Neurologic and Psychologic Aspects of Allergy—Michael Zeller, M.D., Chicago, Ill.
Food and Digestive Allergy (Illus.)—Herbert J. Rinkel, M.D., Kansas City, Mo.
Allergy of the Central Nervous System—T. Wood Clarke, M.D., Utica, N. Y.
Drug Allergy—Jonathan Forman, M.D., Columbus, Ohio.
Pediatric Allergy—Ralph Bowen, M.D., Houston, Texas.
Allergy Elimination Diets for Children, Albert V. Stoesser, M.D., Minneapolis, Minn.
Mold Allergy (Illus.)—Homer E. Prince, M.D., Houston, Texas.
Bronchial Asthma—Leon Unger, M.D., Chicago, Ill.
Physical Allergy—Cecil M. Kohn, M.D., Kansas City, Mo.

The price of the complete set is $3, and that of the Manual of Allergy Laboratory and Diagnostic Procedures is $2. Please mail your check with your order.

AMERICAN COLLEGE OF ALLERGISTS
401 La Salle Medical Building
Minneapolis 2, Minnesota

BOOK REVIEWS

THE RECURRENCE OF REPEATED ERUPTIONS FOLLOWING VACCINE THERAPY (Les éruptions consécutives à l'emploi des vaccins médicamenteux). By Carlos Malbrán, M.D. 72 pages. Paris, France: Librairie Maloine, 1938.

In the preface of this book the author states that he was greatly impressed and encouraged by the excellent work of his teacher, Dr. M. Clement Simon of the Department of Venereal Diseases of Saint-Lazare Hospital in Paris, France.

He states that the skin has been considered for a long time as a symbol of internal reactions, or rather as a "mirror" of the various phenomena that take place in the organism. It is true, he adds, that these skin eruptions have been considered to be not so frequent, yet he believes that, at times, they are so outstanding and obstructing that they are serious enough to scare the patient, materially.

In the first chapter he discusses the different conceptions of the word "vaccine." He mentions the fact that there are many varieties of vaccines, namely: (1) vaccines that are composed of various bacteria killed in several ways, and suspended in physiological normal saline; (2) lipo-vaccines; (3) bouillon-vaccines, which appeared following the work of Pasteur; and (4) anatoxins, emphasized by Ramon.

The author feels that we should adhere to the interpretation of the term allergy as first conceived by Von Pirquet, namely "another reaction"; the reaction of the allergic subject is different than that of the new subject.

The word allergy comprises these three elements: (1) acquired hypersensitiveness, which gives a prematureness to the atypical or allergic reaction; (2) the existence of a relative immunity, which, even though incomplete, persists for a short time; and (3) every allergic state which is determined by the presence of a pre-existing specific inoculation.

In the second chapter he treats of the large and combined erythemas, febrile, that occurred following injections of Propidon, in a case of chronic cervicitis.

In the third chapter he discusses all other observations made by other workers, in this particular field, like Blum (P) and Bralez (J); Ferrabouc, Friess and Rolland (A); Gougerot (R), Barthélemy and Jean Will; Ledo (E); Milan (G); Périn and Huwart (P); Sézary, Dérot and Guédé.

In the fourth chapter he deals with the pathogenic varieties of eruptions which follow vaccine therapy, both from external and internal causes.

He concludes with these remarks:

1. "We propose the following classification, etiopathogenic, for the consecutive eruptions following vaccinotherapy: A. *By an External Cause,* the vaccine itself; technical error, either in its preparation or in its dosage: (a) infectious eruption due to insufficient attenuation of the vaccine, (b) or an eruption of the toxic kind, arising from certain substances found in the vaccine itself, or due to large doses of this latter. B. *By an Internal Cause:* (a) contained in the individual himself, (b) caused by the awakening of latent bacteria, namely, biotropic phenomena ("vaccinotropides"), following vaccinotherapy.

2. "We believe we have underlined the extreme rarity of eruptive accidents following series of vaccinotherapy, a method well used now. But if their frequence is small, its theoretic interest is important for the pathogenic study of certain skin diseases, and even for the general biologic problems.

3. "Our case of combined consecutive erythemas following injections of Propidon is an example of a biotropic eruption ("vaccinotropide"). Whether it is a direct biotropism, giving an atypical erysipelas to streptococci, or an erysipeloid to staphylococcus (Troisier), we do not know."

H.I.S.

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ALLERGY DIAGNOSIS

now simplified!

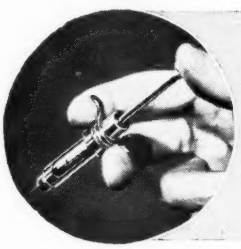
INTRACUTANEOUS TESTING FOR PROTEIN SENSITIVITIES *QUICK, EASY, ACCURATE*

WITH WYETH ALLERGENIC TESTING SET

1. PREPARE SYRINGE:

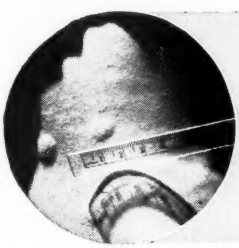
Simply insert TUBEX* of possible exciting allergen into breech-loading syringe—then close breech which locks TUBEX into place.

*REG. U. S. PAT. OFF.



2. INJECT ALLERGEN:

Sterile, unexposed allergen is now ready for intracutaneous testing. Subsequent injections can be made immediately with same needle and syringe by merely flushing cannula with TUBEX of sterile water between tests.



3. READ TESTS...

In just 10 minutes! In fact, intracutaneous tests read sooner, if reaction is positive.

Wyeth

ALLERGENIC TESTING SET

in handsome cabinet, includes breech-loading TUBEX syringe, over 200 TUBEX of essential allergens, one dozen needles, plus other helpful accessories.

A Biological Product of WYETH INCORPORATED, Reichel Division, Philadelphia 3, Pa.

MEAD PRODUCTS
of Interest to Allergists

Nutramigen *1 lb. packages*	A feeding for milk-sensitive infants, which contains a non-antigenic form of nitrogen (Amigen) as the protein component, combined with other food essentials.
Sobee *1 and 4 lb. packages*	A soybean food designed as a substitute for infants exhibiting idiosyncrasy to milk protein.
Pablum *½ lb. and 1 lb.-2 oz. packages*	A palatable mixed cereal food, precooked and dried (needs no further cooking). Furnishes not only high food energy value but also thiamine and riboflavin and calcium, phosphorus and iron.
Pabena *8 oz. packages*	A new form of Pablum, in which the only cereal grain is oatmeal. Has essentially the same nutritional advantages of Pablum, and all of its convenient and economical points.
Mead's Dextri-Maltose with Yeast Extract and Iron *1 and 5 lb. tins*	Supplies, in addition to the carbohydrate value of Dextri-Maltose, thiamine and riboflavin (vitamins B_1 and G).
Mead's Oleum Percomorphum with Other Fish Liver Oils and Viosterol *10 and 50 c.c. bottles* *Boxes of 48 and 192 83-mg. capsules*	A source of vitamins A and D in which not less than 50% of the vitamin content is derived from liver oils of percomorph fishes. Consists of liver oils of percomorph fishes, viosterol, and fish liver oil. Each gram contains not less than 60,000 vitamin A units and 8,500 vitamin D units (U.S.P.).
Mead's Cod Liver Oil Fortified with Percomorph Liver Oil *3 oz. and 16 oz. bottles*	Consists of Mead's Standardized Cod Liver Oil with percomorph and other fish liver oils. Not less than 50% of the vitamin content is derived from percomorph liver oil. Supplies not less than 6,000 vitamin A units and 850 vitamin D units (U.S.P.) per gram.
Mead's Standardized Cod Liver Oil *4 oz., 8 oz. and 16 oz. bottles*	Each gram supplies not less than 1,800 vitamin A units and 175 vitamin D units (U.S.P.).
Mead's Viosterol in Oil *10 c.c. and 50 c.c. bottles*	For disturbances of calcium-phosphorus metabolism. Supplies 10,000 U.S.P. vitamin D units per gram.
Mead's Cod Liver Oil with Viosterol *4 oz. and 16 oz. bottles*	Contains 1,800 vitamin A units and 400 vitamin D units (U.S.P.) per gram.
Mead's Viosterol in Halibut Liver Oil *10 c.c. and 50 c.c. bottles* *Boxes of 25 and 100 capsules*	Supplies 60,000 vitamin A units and 10,000 vitamin D units (U.S.P.) per gram.
Mead's Halibut Liver Oil *10 c.c. and 50 c.c. bottles*	For vitamin A therapy. Each gram supplies 60,000 vitamin A units and 850 vitamin D units (U.S.P.)
Mead's Brewers Yeast Tablets *Bottles of 250 and 1,000 tablets*	For deficiencies of vitamin B complex. Each tablet contains not less than 0.06 mg. thiamine, 0.02 mg. riboflavin and 0.15 mg. niacin.
Mead's Brewers Yeast Powder *6 oz. bottles*	The same product as Mead's Brewers Yeast Tablets but supplied in powder form for use in infant feeding formulas.
Mead's Ascorbic Acid Tablets *Bottles of 50 and 250 tablets*	For prevention and treatment of scurvy. Each tablet supplies 25 mg. of ascorbic acid, the equivalent of 500 International units of vitamin C. Also supplied in 100 mg.
Mead's Thiamine Hydrochloride Tablets *Bottles of 50 and 250 tablets* *0.75 mg. and 3 mg. tablets*	The anti-neuritic factor for prevention and treatment of beriberi and other deficiencies of vitamin B_1. Tablets containing 0.75 mg. thiamine supply 250 International units; 3 mg. tablets supply 1,000 International Units of vitamin B_1.
Mead's Riboflavin Tablets *Bottles of 50 tablets*	Each tablet supplies 1 mg. of riboflavin (vitamin G).
Mead's Niacin Tablets *Bottles of 50 tablets*	For treatment of pellagra. Each tablet contains 20 mg. niacin.

MEAD JOHNSON & CO., Evansville, Indiana, U. S. A.